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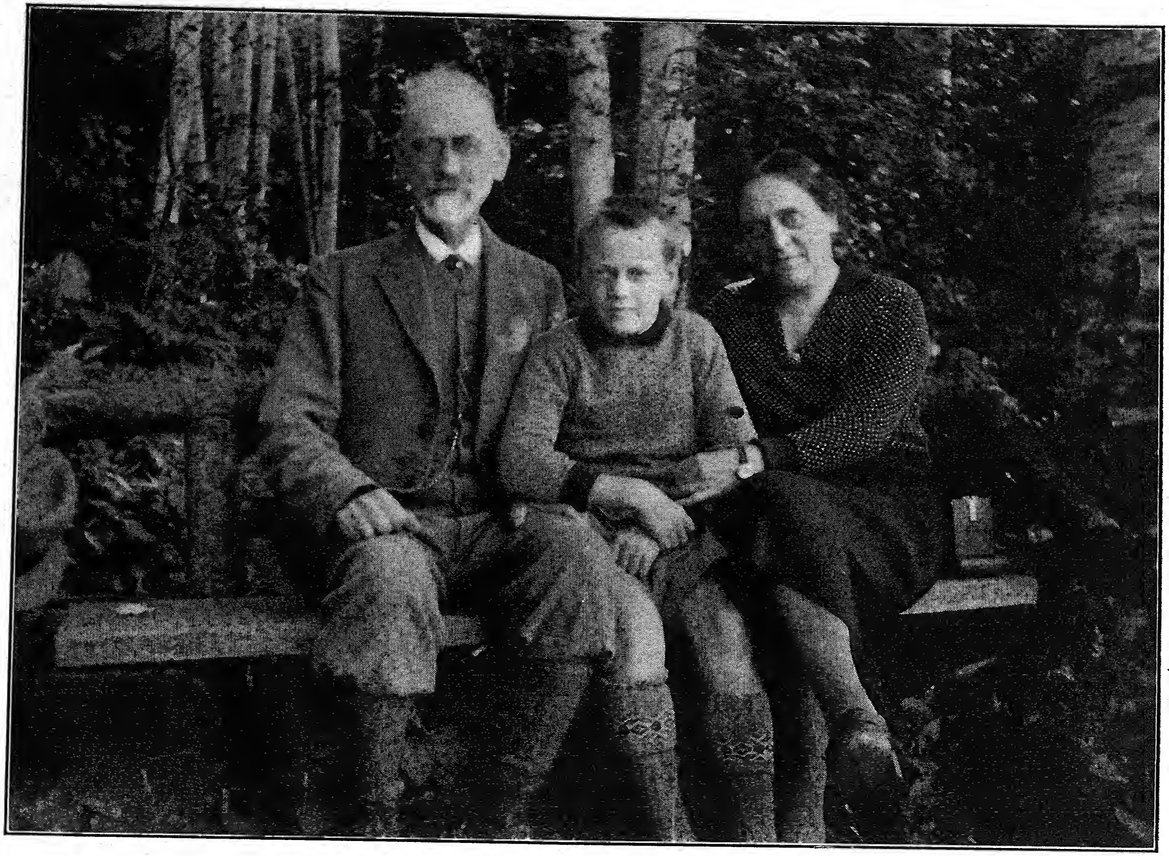

MR. AND MRS. J. E. LANGE AND SON MORTEN

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

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MYCOFLORISTIC IMPRESSIONS OF A EUROPEAN MYCOLOGIST IN AMERICA

JAKOB E. LANGE

(WITH PLATE 1)

Coming over from Denmark late in the summer of 1931, I made a circuit which took me through the majority of the States and parts of southern Canada. Setting out from New York in the middle of August, my itinerary took me through Massachusetts, Vermont, the Adirondacks, Michigan, Minnesota, part of the territory south of Lake Winnipeg to the Canadian Rockies and the Pacific coast from Vancouver to Los Angeles and back to the southeastern States, returning via Washington to New York towards the end of October.

The trip planned in advance by me with the assistance of American friends aroused so much interest that I not only had an excellent opportunity to botanize in some of the best localities, but was able to visit a number of the leading mycologists at home and in their favorite haunts and take part in mycological "conferenciettas" in several scientific centers for the purpose of paving the way for improved Americo-European collaboration in the study of the agarics.

For the overwhelming hospitality and the genuine interest shown me during this highly profitable expedition I cannot too warmly and sincerely thank my American colleagues and friends. Some of my impressions and observations I shall try to set forth.

When a European botanist makes the acquaintance of the plant-life of Northeastern America he cannot avoid being impressed by

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the similarity of the floras on both sides of the Atlantic. To be sure he encounters leading American genera, such as *Aster* and *Solidago,* represented by hundreds of species, which are only scantily represented in the European flora, and vice versa. But apart from these the flora on the American side consists mainly of two types: the *introduced* species and the *parallel* one (*i.e.,* species which, although not identical with the European ones, are so similar that it often requires a keen and practiced eye to distinguish them).

The *introduced* species, although perhaps not very numerous, play a rather prominent part. They are often very common and, as they mainly inhabit cultivated fields, roadsides, and waste places, are rather conspicuous. When strolling along a country lane one cannot avoid seeing everywhere such plants as *Chenopodium album, Sinapis arvensis, Capsella Bursa-pastoris, Cirsium arvense, Plantago major, Dactylis glomerata, Polygonum aviculare* and *Rumex crispus.*

The *parallel* species are likely to attract the newcomer's interest more strongly. They occur in almost all families, and in many cases they are so close to European species that it is difficult to say whether they ought to be characterized as varieties or should be given specific rank. I shall mention only a few as examples (I put the European parallel in brackets): *Populus tremuloides* (*P. tremula*), *Sambucus canadensis* (*S. nigra*), *Plantago Rugelii* (*P. major*), *Parnassia caroliniana* (*P. palustris*), *Populus virginiana* (*P. nigra*), *Larix americana* (*L. europaea*), *Lepidium virginicum* (*L. ruderale*).

These characteristic floristic facts are of course universally known and thoroughly investigated by botanists from either side of the Atlantic. But what about the fungus-flora?

That certain fungi have a universal distribution is a well known fact. These *cosmopolites* are either native (*e.g.,* many coprophilous fungi, such as *Coprini*) or they are introduced from one continent to another, having become established in their adopted home, sometimes even more fully than in their native land. This is true of numerous parasitic forms, such as the potato-blight (*Phytophthora infestans*), the gooseberry-mildew (*Erysiphe Mors-uvae*), and the hollyhock-rust (*Puccinia Malvacearum*).

On the other hand it is also a well established fact that the flora of any continent besides these cosmopolites comprises an element of endemics: species, and even genera, which are exclusively American or European. In the phanerogamic plant world the overwhelming majority of the species are decided endemics. But what about the mycoflora? Is the main body of the American fungus-flora decidedly "American," or does it consist for a large part of cosmopolitan species which are familiar to the European mycologists from of old, and therefore in common parlance are called "European" species? And finally are the specifically American species mainly *parallels,* or are we more likely to meet with species which represent other types, widely separated from those met with in the Old World? In other words, is the American fungus-flora chiefly characterized by *identity, parallelism,* or *incongruity?*

This is a field of investigation which is as yet very incompletely explored. And what is worse, some of the conclusions are of doubtful value. To be sure certain districts of this realm of fungi are comparatively well known and carefully mapped out. This is true of the *parasitic fungi* and also to a certain extent of the suberous and ligneous Polyporaceae. But when we enter the wide land of the agarics (and other fleshy fungi) we at once feel ourselves in a swampy wilderness, where roads are few and the existing landmarks often mere will-o'-the-wisps.

The reasons for this deplorable state of affairs are not far to seek. A direct comparison of European and American finds is not possible: the perishable fruit-bodies would be unrecognizable before reaching the other side of the Atlantic. Cultivation of the American species in European botanical gardens (or vice versa) for the sake of comparison has hardly been attempted, and will probably in most cases be a very difficult undertaking fraught with disappointments. Dried specimens and those otherwise preserved are in most cases of very limited value, and printed illustrations are entirely inadequate for deciding between parallelism and identity.

To these difficulties must be added the one which arises from the inadequacy of the descriptions. The diagnoses of the classical authors are often so defective that it is very difficult if not en-

tirely impossible to identify the species, and the attempts at identification undertaken by modern authors therefore often give contradictory results. The consequence is that when an American mycologist, who knows the "European" species from descriptions only, faces the problem whether a particular fungus, which he finds in Maine or Michigan, is the one named *Agaricus "X"* by Fries, Berkeley, or Quélet, or something else, he will often be at his wit's end and may count on his buttons to settle the case. And the result may be either that he creates a new species, although his specimens were really identical with a European one, or that he dubs his find with one of the classical names, although in truth it is something absolutely different.

A historic example will serve to illustrate: About 1880 Peck, one of the fathers of American mycology, described a mushroom which he referred to Fries' *Agaricus* (*Psalliota*) *campestris silvicola.* Later on he rightly reached the conclusion that it was more closely related to *A. arvensis* than to *A. campestris,* and he therefore altered the name to *Agaricus* (*Psalliota*) *arvensis* var. *abrupta,* which name he in his latest publications modified to *Agaricus* (*Ps.*) *abruptibulba* Peck, thus creating a "new American species." But the fact is, as even a good photograph will prove, that the Peckian species is the very same as the yellowish-white mushroom so frequently met with in any European fir wood, and which by European authors is called *Psalliota silvicola* (Fries) ex Lange or *Ps. arvensis* ex Ricken.

This will serve to illustrate—and similar cases are probably legion—what extraordinary obstacles must be overcome, what knots must be disentangled before we can acquire adequate knowledge in this field, and form well founded conclusions with regard to the problem whether identity, parallelism or incongruity is the most prominent feature of the fungus-floras of the two continents.

My main purpose in visiting America in 1931 was to get by personal observation a firsthand knowledge of this problem. Partly by what I saw myself, and partly by talking it over with American colleagues, I have reached conclusions which, I think, carry us at least a short step forward through the wilderness. But before entering upon a discussion of my results I will give a brief account of my impressions of the localities visited, as they appear to the eye of a Danish mycologist.

As mentioned above, most of my time was spent in the hilly woodlands of Vermont, the Adirondacks (in the northern part of New York State), the more level country around Ann Arbor (Michigan), the low, pine-clad mountains in the lake district southeast of Lake Winnipeg, the Canadian Rockies (Banff and Lake Louise), Vancouver, and the coast ranges and other mountains of Oregon with their vast virginal woods. When I reached California everything was dried up, and not till the middle of October around Washington, on my way back to New York, did I again encounter a rich mushroom vegetation.

To a Danish field-mycologist, accustomed to comparatively small woods and plantations, which everywhere bear the trace of the forester's activity, where stumps are carefully removed and no sick or dead tree allowed to stand, where in short order and uniformity rule supreme, it is an almost overwhelming experience to wander through an American forest, where all kinds of trees can be met growing together, and where fallen decaying trunks and branches, half-hidden in the ground, are the home of innumerable fungi.

First of all, the difference is in the *age* and *kinds* of trees. While in Denmark a plantation or wood generally is made up of thousands of individual trees of the same species and all of one age (having been planted or sown all at one time), and its ground-vegetation of herbaceous plants as a rule likewise is very uniform, the American forest is often a motley collection of different trees, coniferous and frondose (maybe a score of different species to the acre), and the self-sown progeny of the parent trees sprout up everywhere, older and younger together. Under such conditions the whole wood becomes the home of the entire fungus-flora, while in Denmark most of the species are strictly localized. An example will serve to characterize the difference:

Lactarius deliciosus—the well known edible milksop—here in Denmark is strictly confined to our plantations of *Picea.* But more than that, it is confined to plantations of a certain age (rather young). If anyone has the opportunity to watch the growth and development of such a plantation, he will therefore see *Lactarius deliciosus* make its appearance and in the course of a series of years disappear again, when the trees completely overshadow the ground and quell the grassy vegetation below. But in an Ameri-

can forest no such periodicity is likely to occur; it is not a plantation of a certain age, but a collection of trees of all ages.

The fungus-flora under such conditions is very rich. In good seasons the American woods are a true Eldorado for the mushroom-hunter. This "embarras de richesse" is likely to overpower the botanist from foreign parts. What is particularly bewildering to the Danish field-mycologist is the apparent lack of any intimate relation between certain species of fungi and certain species of trees. Of course even the Danish fungus-flora abounds in species which are to be met with under all and any conditions, in all kinds of woods, such as *Hypholoma fasciculare, Inocybe geophylla,* and *Laccaria laccata.* But a very large number are strictly attached to certain trees, being either fagophilous, pinophilous, laricophilous, or at least confined to coniferous or frondose woods. Thus it cannot escape the attention of a mycologist who studies the Danish flora that, for instance, *Boletus bovinus, Limacium hypothejus, Gomphidius viscidus* and *G. roseus,* are strictly confined to our pine woods, while *Tricholoma psammopodum* and *Boletus elegans* grow under *Larix.* But in an American forest, where all kinds of trees are often crowded together, pinophilous and dryophilous species may be met with on the same spot. No wonder that the American mycologists, when describing the habitat of a certain fungus, often resort to such vague expressions as "in woods," "in shady places," "under trees" and the like, while a careful European mycologist is likely to be more precise in this respect. Still, although more difficult to ascertain, the connection between a fungus and its specific tree can generally be traced even in American woods, at least in those cases where the attachment is absolute.

Altogether such a mycological survey as mine, extended over vast territories, rich on contrasts and extremes, is a unique experience, stimulating and inciting to a more thoroughgoing study of this part of the vegetable kingdom.

PARALLELISM AN IDENTITY OR INCONGRUITY

Returning to the main subject: What then are the conclusions, if any, with regard to the similarity or dissimilarity of the American and European flora of agarics (and other hymenomycetes), to

be gathered from these forays and mycological discussions? Of course even such a ten weeks' trip is by far too short a time for final conclusions. Nevertheless I make bold to state as a preliminary result that the difference between the European and the American fungus-flora is not nearly so great as might be expected from American monographs and floras.

If one turns to such publications, of later years, the general impression will be that the proportion between exclusively American species and "Europeans" is about 7:3. But not only in the eastern States but also in the North and West, wherever I gathered a fairly large number of species, I found more nearly the inverse proportion: 70 per cent which were known to me from the European side of the Atlantic, against 30 per cent specifically American. Wherever a European mycologist may go in American woods he will meet with species familiar to him. Generally the main aspect of the American fungus-flora will be very "European." Or to put it more correctly: *The American mycoflora has more of a cosmopolitan stamp than of an exclusively American one.*

And this main conclusion will hardly be modified to any considerable extent by further and more thoroughgoing investigations. To be sure it is not unlikely that a number of rare species, which do not play a prominent part on the mycological stage and which to a large extent will have escaped my eye, are exclusively American. But on the other hand it is also highly probable that species which are now counted exclusively American will be met with also on the European side of the Atlantic by more careful investigators. In fact recent European publications have brought to light a considerable number of such cases. I myself have found *Pleurotus Rhacodium* (Berk. & Curt.), and *Lactarius griseus* (Peck) has been met with in France. Evidently "the pond" is too narrow to bar out such peregrinating species.

New evidence for the preponderance of the cosmopolitan species over the exclusively American ones is also brought out in a recent work (P. F. Shope: The Polyporaceae of Colorado). Although the climatic conditions and the phanerogamic flora of Colorado are very different from the European type, only 27 per cent of the Polyporaceae enumerated are exclusively American. The fact that most *Polypori* can be identified in a dried condition and their

identity with European species therefore more easily ascertained, will account for this result.

But what about *parallelism* in the world of fungi?—It goes without saying that the more numerous the truly Americo-European species are the less will be the chance of meeting with parallels. Still their number seems to be not at all insignificant. The most prominent and best known of these parallel species is the orange-yellow "fly-mushroom." While in Europe *Amanita muscaria* is almost always bright scarlet (a brown variety is mentioned by Fries but seems to be exceedingly rare), all over the Eastern States a similar species but with a clear orange-yellow hue occurs. This American form also differs slightly from the European one by a faint ochrey tinge of the white dots on the cap (which in the European form are pure white). Whether this American type should be called *A. muscaria* var. *americana* or raised to specific rank is a matter of taste. The existence of this orange-colored American form is the more remarkable because the scarlet European type also occurs in certain parts (*e.g.,* the eastern Canadian provinces and Oregon, where it is said to attain gigantic dimensions).

Also the European *Amanita virosa* has a parallel form *A. bisporigera,* which differs from *A. virosa* by its much smaller dimensions and particularly in its two-spored basidia. It is also very close to *A. verna,* which species—while rare in North Europe—seems to be very common in America (eastern United States), figuring there as a kind of substitute for *A. phalloides,* which seems to be almost unknown in America, while it is very common with us.

Whoever knows the rare species of *Lactarius, L. scrobiculatus* (characterized by the latex quickly changing into chrome-yellow), and in an Adirondack woods finds a *Lactarius* of exactly the same general appearance but with the latex turning lilac instead of yellow, will feel inclined to count it an American parallel, if this lilac-milked form had not been encountered half a century ago in the Bavarian mountains in Germany and named *L. repræsentaneus.* But what a striking example of the wide distribution of a rare species! One of the most remarkable parallels in America, as well in the southern as in the northern states, is *Lactarius de-*

ceptivus, which bears a striking likeness to the European *L. piperatus,* but is easily recognized—when young—by having an almost cottony velum edging the cap.

Also the " edible milksop " *Lactarius deliciosus* (which by the way is rather common in the United States) has an exclusively American parallel, *L. subpurpureus.* The shape of the cap, the size and texture are exactly alike, but the milk of *L. subpurpureus* is dark dingy purple, and the original color of the cap and gills, which changes to dull green with age as in *L. deliciosus,* is a dingy flesh color, while *L. deliciosus* is tile-red with carrot-red latex.

A fungus-foray in America is an excellent training for a mycologist desirous of sharpening his eye for slight differences and minute characteristics. Often the parallel species are so close to each other that anyone not absolutely wide awake will fail to distinguish them. A very characteristic example may be cited: Every European mycologist knows the large *Mycena,* so common in our beech woods, which is called *M. pelianthina* and which is so easily recognized by the blackish-purple edging of the gills. This species is mentioned in several American lists. And to be sure the very first day I botanized in Vermont I encountered a *Mycena* answering almost exactly to the description of *M. pelianthina.* Still it struck me that the color was slightly different; the entire plant, but particularly the stem, had a flush of amber-yellowish, which never occurs in the European *M. pelianthina.* And when further investigation showed that the spores were about 1½ times the size of those of *M. pelianthina,* it was clear to me that the American form would have to receive a specific name (I propose **M. pseudopelianthina** nom. nov.) and represented a case of close parallelism.

Also a *Lepiota* very close to the common European-American species *L. clypeolaria* was collected in the same parts. It differs from the genuine *L. clypeolaria* by being considerably shorter and smaller and—what is more important—by having quite small oval spores ($6 \times 4\,\mu$), while those of *L. clypeolaria* are almost fusiform and about three times as long.

The genus *Pluteus* affords several cases of particular interest. In the Adirondacks I saw a single specimen of the brilliant scarlet species which was named *P. caloceps* by Atkinson. Except for

the bright vermilion color it is in every respect like the European *P. leoninus* and might be taken for an American "parallel"—if the very same scarlet form had not been recorded in Europe (England and Denmark), and figured in Cooke's Illustrations, under the name *P. leoninus* var. *coccineus.* Another possible parallel to *P. leoninus* is a tiny little yellow species called by American mycologists *P. admirabilis.* I have never seen the European *P. leoninus,* which is said to be somewhat larger; but it appears to me rather doubtful whether *P. admirabilis* really deserves specific rank.

The European species *Pholiota erebia* (which even in Europe has been dubbed with a number of names, the most awkward being that of "*Armillaria denigrata*") apparently has a lot of American substitutes. Peck has a long series of names, such as *Pholiota aggericola* and *P. indecens.* But as far as I can see none of these differ materially from the various forms of *Pholiota erebia* occurring on the European side of the Atlantic.

One of the most peculiar cases of parallelism is the existence in America of a phosphorescent form of *Panus stipticus.* While there is no record—as far as I know—of any phosphorescence in the European form, the American one is renowned for its bright noctilucency. I myself encountered this phosphorescent form in North Carolina (Cherokee Co.) in 1927. When seen by daylight the specimens were exactly like those so commonly collected in Denmark. Whether the phosphorescence is a real specific difference or can be accounted for by atmospheric conditions (or bacteria) remains to be decided by further investigations. Finally I may also mention that the genus *Pleurotus* includes an American parallel. *P. Rhacodium* differs from *P. applicatus* only in the black plushy coating on top of the pileus. But this species also has in recent years been found in Europe (Denmark).

Altogether the number of true parallels substituting European forms in the Western hemisphere, and not found on the European side of the Atlantic, seems to be rather limited.

Evidence is as yet far too incomplete to draw any final conclusions with regard to the problem of parallelism. But to my mind the facts point in a certain direction: Everywhere in the vegetable and animal kingdom new "small species" or varieties seem to arise by "*mutation*" (sudden leaps or sidesteps from the straight

path of heredity). If such new forms be equally well or better adapted to the natural conditions in the country where they arise, they may establish themselves there or even become the exclusive possessors of the territory hitherto occupied by the parent species. If such new forms have limited means of dispersal they will become local species. If adapted for wide dispersal, they may gradually spread over unlimited areas.

Supposing such mutations occur among fungi, which are easily dispersed, there is an overwhelming probability that the new species in the course of time will invade adjacent countries, thus reestablishing the temporarily disturbed identity of the floras. And the world wide distribution of a great number of agarics shows that even the Atlantic is too narrow to prevent such migration. Such cases as those mentioned (*Pluteus caloceps, Pleurotus Rhacodium,* and *Lactarius repraesentaneus*) may be explained this way, whether their origin be on this side of the Atlantic or in America. And it is not at all unlikely that such species as *Lactarius subpurpureus* and *Mycena pseudo-pelianthina,* hitherto only known from the Western hemisphere, will in time be discovered also somewhere in the Old World.

By far the greatest distinction in the world of fungi is not between a *western* and an *eastern* flora, but between southern and northern. The distinctive features of the tropical and subtropical mycoflora are very pronounced, subcoriaceous agarics like *Marasmius* and *Lentinus* abound, as well as Phalloids, in the south; and even the southern temperate climates have a flora of their own. In Europe we have a considerable number of Mediterranean species which never (or very rarely) overstep the barrier of the Alps. Other species (such as *Amanita caesarea*) have their northern limit in central Germany, or may extend to northern Germany or southern Denmark (species of truffels, *Amanita solitaria,* etc.). On the other hand certain northern species, which abound from the Rocky Mountains to the Scandinavian subarctic zone, such as *Stropharia depilata,* do not reach so far south as Germany. Similar cases may probably be cited from America.

But in addition to the direct effect of the climatic conditions, the fungus-flora evidently is influenced by the phanerogamic vegetation, more especially by the presence or absence of certain trees to

which the particular species is attached. This may account for a good deal of the incongruity of the floras of Europe and America, and those of the eastern and western United States.

That the American *Agaricus*-flora, in spite of the super-abundance of Americo-European species, comprises a great number of clearly distinct and often very characteristic species, is evident. To a European mycologist it adds a certain zest to the joys of the day in an American wood to meet with such extraordinary types as *Amanita flavoconia, Clitocybe illudens, Laccaria ochropurpurea, Collybia myriadeophylla, Mycena Leajana* and *M. aurantidisca, Volvaria pubescentipes, Clitopilus abortivus, Entoloma salmoneum* and *E. strictius, Pholiota albo-crenulata* and *P. erinacella, Russula compacta, Marasmius siccus* and *M. pulcheripes, Boletinus pictus,* and *Craterellus Cantharellus.*

But stronger and more lasting than any other impression is the evidence of the wonderful cosmopolitanism of the Agarics. When you have once found, in a Danish *Sphagnum*-bog, a few specimens of the "new" species *Stropharia psathyroides* Lange, it gives you a shock to meet with the very same plant in a bog in Oregon, near the Pacific Coast—and only an hour later to come upon *Lepiota cygnea* Lange, of which the only known specimens were hitherto those gathered in 1925, a few miles from my Danish home!

Who can trace the aerial course of the spore?!

THE HYDNACEAE OF IOWA. II. THE GENUS ODONTIA

L. W. Miller

(with plates 2 and 3)

Odontia is the only strictly resupinate genus of the Hydnaceae characterized by the presence of cystidia. *Steccherinum ochraceum, S. lacticolor* and *S. setulosum* have cystidia and may occur resupinate but these can be distinguished generally by their more coriaceous texture and larger size. Cystidia are readily separated from gloeocystidia, setae and conducting organs but often are distinguished with difficulty from the sterile hymenial organs known as paraphyses. Sterile hymenial organs which are readily distinguished from basidia are here treated as cystidia. A cystidium is generally regarded as the specialized end of an undifferentiated hypha and typically has thickened walls, no conspicuous content and is often incrusted with granular material (Overholts, *1929*). It seems unwise arbitrarily to distinguish between the unspecialized hyphae and typical cystidia projecting at the crest of the tooth in many species of *Odontia,* particularly since this character may vary in a given species.

Key to the species of Odontia

1. Cystidia elongated, fusiform or cylindrical, usually strongly incrusted and with thickened walls. .... (2)
1. Cystidia not as above, variable; if greatly elongated either smooth or only slightly incrusted, or sometimes heavily incrusted, thin-walled, and obscured in axial bundles. .... (7)
    2. Cystidia distinctly fusiform. .... (3)
    2. Cystidia long, mostly cylindrical. .... (4)
3. Cystidia sometimes arising from a specialized, septate, incrusted, axial hypha; spores short cylindrical, $3.5–5 \times 1.5–2\ \mu$. .... 1. *O. hydnoides.*
3. Cystidia not arising from a specialized hypha; spores oblong, $4.5–5.5 \times 3–3.5\ \mu$. .... 2. *O. Queletii.*
    4. Cystidia largely restricted to the apex of the tooth, usually 1–6 occurring in each tooth, septate, smooth, then heavily incrusted; spores mostly $9 \times 4–5\ \mu$. .... 6. *O. setigera.*
    4. Cystidia numerous, projecting from the sides and apex, not septate; spores not exceeding $5 \times 3\ \mu$. .... (5)

5. Fructification separable, with numerous rhizomorphic strands; teeth short, hispid; spores 3.5–4.5 × 2–3 μ. ....3. *O. fimbriata.*
5. Fructification adnate, without rhizomorphic strands; teeth slender. ..(6)
   6. Ceraceous; hyphae 2–4.5 μ, with few cross-walls; spores 4–5 × 2–3 μ. ....4. *O. ciliolata.*
   6. Floccose, with a fragile, pruinose hymenial surface; hyphae 5–7 μ, with many septa; spores 3–3.5 × 1.75–2.25 μ. ....5. *O. laxa.*
7. Cystidia long, cylindrical, relatively undifferentiated, smooth, in compact or loose terminal tufts; spores long cylindrical. ....(8)
7. Cystidia similar or otherwise; spores spherical to short cylindrical. .(11)
   8. Cystidia agglutinated by a resin-like material into a more or less compact, cylindrical, viscid fascicle; spores 5–7 × 1–1.5 μ. 10. *O. sudans.*
   8. Cystidia in loose tufts, little more than slightly swollen hyphae projecting at the apex of the tooth. ....(9)
9. Subceraceous, whitish; spines conical, minute; basidia 12–18 × 4–5 μ; spores 6–7 × 2–2.5 μ, flattened on one side. ....7. *O. cristulata.*
9. Subfloccose, near cinnamon-buff; spines larger. ....(10)
   10. Basidia 10–20 × 4–5 μ; spores 7–9 × 1.5–2 μ, curved. 8. *O. alutacea.*
   10. Basidia 18–35 × 5–7 μ; spores 7–10 × 3–4 μ; slightly curved. 9. *O. subalbicans.*
11. Cystidia not restricted to the apical portions of the teeth, consisting of subulate, paraphysoid structures or with enlarged or incrusted terminations. ....(12)
11. Cystidia more or less restricted to the apical or outer portion of the teeth. ....(14)
   12. Cystidia incrusted or bearing crystalline material at the ends. (13)
   12. Cystidia subulate, thin-walled, not incrusted, of the same diameter as the basidia, spores 6–8 × 3–4 μ. ....16. *O. crustosa.*
13. Cystidia terminated by a globose enlargement, usually with radiating crystals; spores 4.5–6 × 2.5–3 μ. ....15. *O. bicolor.*
13. Cystidia consisting of constricted hyphal ends which are incrusted for a distance of 8–12 μ; spores 5–6 × 4–5 μ. ....14. *O. arguta.*
   14. Cystidia incrusted, thin-walled and relatively unspecialized, arising singly or in compact bundles at the apical region of the tooth. The incrusted fascicles or individual cystidia are made more or less conspicuous in a KOH solution. ....(15)
   14. Cystidia smooth or occasionally with scattered crystalline material, often in loose terminal tufts. ....(18)
15. Teeth irregular, obtuse, terminating in white, divided tips. ....(16)
15. Teeth entire, uniformly conical or cylindrical, often with pointed tips. (17)
   16. Fructification honey yellow; teeth rigid, strongly hispid at the apex; spores 6–9 × 3.5–5 μ, faint yellow in mass. ....18. *O. livida.*
   16. Fructification cream buff; teeth short, weakly divided into whitish processes; spores 4–6 × 3–3.5 μ. ....17. *O. crustula.*

17. Fructification dark-gray to burnt umber when fresh, cinnamon-buff to fuscous-black in the herbarium; spores 4.5–6 × 2–3 μ. . 19. *O. fusco-atra.*
17. Fructification mustard yellow to tawny, turning purple upon contact with KOH; spores 4.5–6 × 2–3.5 μ. .... 20. *O. uda.*
    18. Fructification soft, floccose, loosely adnate. .... (19)
    18. Fructification crustaceous, subceraceous or ceraceous, adnate. (20)
19. Teeth bristly on the sides and at the apex, 4 mm. or less in length; cystidia 3–9 μ in diameter, numerous, long cylindrical, thick-walled; spores 4–7 × 2.5–4.5 μ. .... 11. *O. barba-jovis.*
19. Teeth with one or more pointed terminal tufts of cystidia, minute; cystidia 2.5–4 μ in diameter, long cylindrical; spores 4–6 × 3–4 μ. 12. *O. stipata.*
    20. Fructification mars yellow to mars brown; teeth coalesced, and strongly fimbriate at the apex; spores 3–4.5 × 1.5–2.5 μ. (*Oxydontia stenodon*).
    20. Cartridge buff to light ochraceous-buff; teeth 1.5 mm. or less in length, variable, subulate, cylindrical or spathulate; spores 4–6 × 2.5–4 μ. .... 13. (*Radulum?*) *spathulatum.*

1. ODONTIA HYDNOIDES (Cooke & Massee) v. Höhn. Akad. Wiss. Wien. Sitzungsber. **118**: 817. 1909. (PLATE 2, FIG. 1.)

Peniophora hydnoides Cooke & Massee, Jour. Linn. Soc. **25**: 154. 1888.

Odontia conspersa Bres. Accad. Sci. Lett. Rovereto III. **3**: 100. 1897.

Peniophora crystallina v. Höhn. & Litsch. Akad. Wiss. Wien. Sitzungsber. **116**: 828. 1907.

Effused, thin, adnate, ceraceous, then farinaceous, white to cinnamon-buff; margin indistinct; teeth 0.5 mm. or less in length, subulate to cylindrical, variable, generally slender and fragile, subdistant, with prominent projecting cystidia at the sides and crests; hyphae 2–3 μ in diameter, indistinct, no clamp connections seen; cystidia 25–70 × 8–14 μ, subulate or fusiform, walls thickened, incrusted, occasionally more or less fascicled about an axial, incrusted and septate cystidium, 8–10 μ in diameter; basidia 8–15 × 3–5 μ, subulate, with 4 sterigmata; spores 3.5–5 × 1.5–2 μ, short cylindrical, slightly depressed on one side, smooth, hyaline.

This species is identified by its extremely thin fructification, slender, fragile spines, characteristic cystidia and the spore characters. It is separated from *Odontia Queletii* by its more slender teeth and smaller, cylindrical spores. The peculiar axial row of large incrusted cells in a single tooth around which the numerous

subulate or fusiform cystidia are sometimes arranged, is a helpful character. A careful free-hand section or the slight crushing of a spine will reveal this character. Von Höhnel has indicated that this species may at times be nearly devoid of teeth.

This species does not agree with the original description and figures of *Peniophora hydnoides* Cooke & Massee. Von Höhnel (1909), however, has studied the original specimen and reports that it represents the same species as *Odontia conspersa* Bres. and *Peniophora crystallina* v. Höhn. & Litsch. Iowa specimens agree with Bresadola's description of *Odontia conspersa* and are identical with an authentic specimen from Bresadola at The New York Botanical Garden. The synonymy of these two names is quite generally accepted in Europe. Specimens of *Odontia conspersa* from Bourdot and of *Odontia hydnoides* from Litschauer have also been examined.

This fungus is fairly common in Iowa from June to November on decayed wood of coniferous and frondose species. I have seen no report of its previous occurrence in North America. However, I have seen several specimens at The New York Botanical Garden labeled *Odontia glauca* Ell. & Lang. from Louisiana and an undetermined specimen from Minnesota in the mycological herbarium at the University of Iowa.

2. ODONTIA QUELETII Bourd. & Galz. Bull. Soc. Myc. Fr. **30**: 270. 1914. (PLATE 2, FIG. 2.)

Odontia farinacea Quél. Fl. Myc. Fr. 435. 1888.

Fructification ceraceous or subcrustaceous, very thin, not cracking, white to cinnamon-buff; margin absent, not distinct or narrowly limited and fibrillose; teeth scattered or confluent in groups, subulate to short cylindrical, obtuse, fimbriate; hyphae 2.5–5 μ in diameter, with thin walls, fragile, indistinct; cystidia 40–100 $\times$ 8–16 μ, with thick walls, incrusted, usually fusiform, occasionally cylindrical or clavate at the crest, numerous, projecting prominently, subimbricated in the teeth; basidia 15–30 $\times$ 3.5–5 μ, cylindrical-clavate, spores 4.5–5.5 $\times$ 3–3.5 μ, oblong, smooth, hyaline, 1–2 guttulate.

The description of Bourdot and Galzin and in part that of Quélet indicates a crustaceous fructification which cracks upon drying, more crowded spines, smaller cystidia and does not in-

clude guttulate spores. These differences may not be fundamental and since the species is quite distinct otherwise, the determination is probably accurate. Furthermore, an authentic specimen from Bourdot at the Farlow herbarium resembles our Iowa collections externally and microscopically. *Odontia Queletii* and *Odontia hydnoides* are quite sharply distinguished from other *Odontia* species by the numerous, projecting, thick-walled, incrusted and fusiform cystidia and by the small spores. *Odontia Queletii* is separated from the latter by the stouter and less crowded teeth and by the character of the cystidia and spores. The young growing borders of the fructifications spread out as a very thin layer which is almost invisible under the lens. In these areas the cystidia stand out as prominent upright structures.

Collected four times on deciduous wood near Milford, Iowa, in June and July. Apparently not previously reported from the United States.

3. ODONTIA FIMBRIATA Fries, Epicr. 529. 1838. (PLATE 2, FIG. 4.)

Hydnum fimbriatum Pers. ex Fries, Syst. Myc. **1**: 421. 1821.

Mycoleptodon fimbriatum (Fries) Bourd. & Galz. Bull. Soc. Myc. Fr. **30**: 276. 1914.

Gloiodon fimbriatum (Fries) Donk, Ned. Bot. Ver. **1**: 79. 1930.

Entirely resupinate, effused, membranaceous, coriaceous, separable, surface plainly marked by intricately branching, rhizomorphic strands, vinaceous-buff to fawn colored; border fibrillose and rhizomorphic; teeth wart-like, short, hispid at the crest, usually subdistant, more crowded over the rhizomorphic strands; hyphae 2.5–4.5 μ, mostly thick-walled, with few cross-walls and no clamp connections, or thin-walled, indistinct and with occasional clamp connections in the subhymenial region; cystidia 50–80 $\times$ 7–9 μ, clavate-cylindrical, obtuse, thick-walled, incrusted, greatly elongated; basidia 12–20 $\times$ 3–5 μ, clavate, with 4 sterigmata; spores 3.5–4.5 $\times$ 2–3 μ, elliptical, smooth, hyaline.

This species is recognized by its cinnamon color, separable membrane and much branched rhizomorphic strands. The coriaceous texture and the character of the cystidia show similarity to *Steccherinum ochraceum* as indicated by Bourdot and Galzin who

have placed the two species together in the genus *Mycoleptodon. O. fimbriata* is a typical *Odontia* except in its coriaceous fructification and this character alone does not seem to be a sufficient reason for placing it in another genus.

Abundant in Iowa. Collected from June to December on frondose species, particularly oak. Reported from central and eastern United States.

4. **Odontia ciliolata** (Berk. & Curt.) comb. nov. (PLATE 2, FIG. 5.)

Hydnum ciliolatum Berk. & Curt. Jour. Bot. & Kew Misc. **1**: 235. 1849.

Effused, ceraceous, thin, adnate, cracking in drying, light ochraceous-buff; border not differentiated or rarely fibrillose; teeth 0.7 mm. or less, slender, crowded, hispid, extending to the margin; hyphae 2–4.5 μ, thick-walled, with few cross walls, or thin-walled and with occasional clamp connections in the subhymenial layer; cystidia 35–60 $\times$ 5–12 μ, subclavate or subulate, obtuse, thick-walled, incrusted; basidia clavate with 4 sterigmata; spores 4–5 $\times$ 2–3 μ, elliptical, smooth, hyaline.

Two specimens of *Hydnum ciliolatum* Berk. & Curt. were examined at The New York Botanical Garden. One was determined by Cooke. The other Banker compared with the type at Kew and marked "identical." Berkeley's original description also indicates the accuracy of their determinations. These and the Iowa specimens seem closely related to *Odontia fimbriata,* and microscopically are practically indistinguishable. However, this species differs in the adnate, ceraceous and lighter colored fructification, the relatively undifferentiated margin and in the character of the teeth.

Two specimens from Iowa were collected in March and in August on much decayed wood of frondose species. Its occurrence is reported from several scattered localities in the eastern United States.

5. **Odontia laxa** sp. nov. (*laxa,* loose). (PLATE 2, FIG. 6.)

Resupinata, tenuis, subadnata, floccosa, ex albida cremea; aculei 0.7 mm., subulati vel cylindracei, gracile; hyphae 5–7 μ, uniformiae, septatae, subhymeniales tenues, non nodoso-septatae; cystidia 40–75 $\times$ 5–10 μ, cylindracea,

incrustata; basidia 7–9 × 3–4 μ; sporae 3–3.5 × 1.7–3.3 μ, ellipsoideae, leves, hyalinae.

Resupinate, very thin and fragile, loosely adnate, soft, floccose. with a pruinose hymenial surface, white to pinkish buff; margin floccose or thin and appressed, sometimes fibrillose, slightly darker in color; teeth subulate to cylindrical, very slender, with prominent cystidia at the sides and the crests; hyphae of the subhymenial region and in the axial portions of the teeth 5–7 μ in diameter, uniform, thick-walled, septate, branching at wide angles, without clamp connections, hyaline or slightly yellowish and often incrusted, becoming more compact, smaller, hyaline, thin-walled and sometimes obscured by granular material in a thin hymenial layer; cystidia 40–75 × 5–10 μ, numerous, consisting of the cylindrical ends of the coarse hyphae in the axial portion of the tooth, projecting, strongly incrusted; basidia 7–9 × 3–4 μ, indistinct; spores 3–3.5 × 1.75–2.25 μ, ellipsoid, attenuate, hyaline.

A specimen labeled *Hydnum fascicularia* Berk. & Curt., on elm, July 30, 1893, in the Morgan collection in the mycological herbarium of the State University of Iowa and presumably from Ohio is identical with Iowa material. A portion of the Morgan specimen was compared with the type of *Hydnum fascicularia* at Kew by Miss Wakefield who reports that it is certainly not the same. Since the Morgan collection is considerably larger than the single Iowa specimen it is here designated the type of *Odontia laxa*. This species has cystidia similar to those in *O. fimbriata* and *O. ciliolata* but is readily distinguished by the floccose texture, the large, uniform hyphae and the smaller spores.

Apparently rare in Iowa. On a fragment of bark near Wellman, Iowa. October.

6. **Odontia setigera** (Fries) comb. nov. (PLATE 2, FIG. 3.)

Thelephora setigera Fries, Elench. Fung. **1**: 208. 1828.

Kneiffia setigera Fries, Epicr. 529. 1838.

Corticium myxosporum Karst. Medd. Soc. Faun. Fl. Fenn. **9**: 53. 1882.

Odontia acerina Peck, Ann. Rep. N. Y. State Mus. **53**: 847. 1900.

Peniophora setigera (Fries) v. Höhn. & Litsch. Ann. Myc. **4**: 289. 1906.

Fructification resupinate, effused, adnate, at first thin, arachnoid under the lens, then thickened, subfloccose to subceraceous, sometimes cracking, white to pinkish buff; margin similar, white; teeth short, conical, blunt, weakly hispid at the apex; hyphae 2–5 μ in diameter, with numerous clamp connections, loosely arranged; cystidia 25–75 $\times$ 6–11 μ, cylindrical, sometimes tapering, septate, with clamp connections, projecting prominently near the apex of the teeth, at first naked, becoming incrusted with coarse oxalate crystals; basidia 15–35 $\times$ 5–8 μ, clavate, with 4 sterigmata; spores 6–12 $\times$ 3.5–6 μ, mostly 9 $\times$ 4–5 μ, ellipsoid to cylindrical, smooth, hyaline, often guttulate.

This species is generally referred to *Peniophora* in Europe. Smooth forms have been reported, but these are apparently unusual. A more or less distinct papillose or warty hymenium is present in our many Iowa specimens and in the dozens of specimens from Europe and North America, which I have examined at The New York Botanical Garden and at the Farlow herbarium. The odontoid character of the fructification is also shown by the usual position of the cystidia at or near the apex of the warts. A cross-section through a tooth of *Odontia granulata*[1] (= *O. setigera*) with a single cystidium is shown by Overholts (1929) on plate 2, figure 5. In forms with less pronounced warts the cystidia appear more scattered but this is not unusual in other species of *Odontia.* If the present distinctions between *Peniophora* and *Odontia* are to be maintained this species seems clearly to belong to the latter genus.

Odontia setigera may be distinguished from *O. cristulata* by the larger, more conspicuous and less fascicled cystidia and by the characteristic spores.

Very abundant in Iowa on wood or bark of oak and many other frondose and coniferous species. Collected from June to December. Reported from various localities in the central and eastern portions of the United States.

7. ODONTIA CRISTULATA Fries, Epicr. 529. 1838. (PLATE 2, FIG. 10.)

Effused, thin, soft subceraceous, slightly cracking and pruinose, adnate, white to cream color; margin whitish, floccose or pruinose;

[1] *Odontia granulata* is a name suggested by Burt but apparently never published. References have been made to it in literature by Lloyd and Overholts. It is *O. setigera* as here understood.

teeth subdistant, conical, very short and fragile, fimbriate at the crest; hyphae 2.5–5 μ in diameter, loosely interwoven, dividing upward and becoming quite compact in the hymenium, with numerous clamp connections; cystidia only slightly differentiated, often irregular in shape, loosely fascicled at the apex of the teeth, with spherical clusters of crystals, with or without cross-walls; basidia 12–18 × 4–5 μ, clavate, with 2–4 sterigmata; spores 6–7 × 2–2.5 μ, cylindrical, flattened on one side, smooth, hyaline, often 1–2 guttulate.

Bresadola and Bourdot and Galzin give the spore size as 8–10 × 3.5–4 μ. However, a specimen of *O. cristulata* Fries from Bourdot at the Farlow herbarium resembled the Iowa specimen externally and seemed to be identical in microscopic structure. This species closely resembles *O. setigera* in external appearance but has less conspicuous cystidia and apparently smaller spores.

Collected only once on much decayed deciduous wood in November at North Liberty, Iowa. No record of its previous occurrence in the United tates has come to my attention.

8. ODONTIA ALUTACEA (Fries) Bourd. & Galz. Hym. Fr. 422. 1927. *non* Bres. (PLATE 2, FIG. 9.)

Hydnum alutaceum Fries, Syst. Myc. **1**: 417. 1821.

Kneiffia stenospora Karst. Hedwigia **25**: 231. 1886.

Resupinate, effused, adnate, thin, loose and floccose, near cinnamon-buff; margin similar; teeth scattered or crowded, conical, pointed or slightly fimbriate; hyphae 2.5–5 μ in diameter, thick-walled, clamp connections numerous; cystidia in terminal tufts and widely scattered along the sides, little differentiated, not incrusted, with or without cross-walls, obtuse; basidia 10–20 × 4–5 μ, with 4 sterigmata; spores 7–9 × 1.5–2 μ, cylindrical, curved, smooth, hyaline.

Bresadola (1897) and probably also Quélet (1888) applied the name *Odontia alutacea* (Fries) to a form of *Odontia arguta* (Fries) Quél. Bourdot and Galzin (1927) report receiving a fragment of an authentic specimen of *Hydnum alutaceum* Fries from Romell. They therefore base their conception of the species on this specimen. My specimen agrees entirely with their description and with an authentic specimen of *Kneiffia stenospora* from Karsten at The New York Botanical Garden, which Bourdot and Galzin cite as a synonym. Three specimens of *O. alutacea* re-

ceived from Litschauer also agree closely with our Iowa collection. The floccose texture and long, cylindrical spores are very characteristic.

One specimen on oak was collected in Iowa, September, 1931. Bourdot and Galzin report a specimen from Lloyd (n. *09130*) but do not state that it was collected in the United States. It is doubtful whether the other records of *Hydnum* (*Odontia*) *alutaceum* Fries in the United States refer to the same species as here defined.

9. ODONTIA SUBALBICANS Pers. ex Bres. Ann. Myc. **1**: 87. 1903. (PLATE 2, FIG. 8.)

Hydnum granulosum var. *albicans* Pers. Myc. Eu. **2**: 184. 1825.

Effused, floccose, with a thin subceraceous hymenium which is easily crumbled when dry, loosely adherent, light pinkish cinnamon to cinnamon-buff; border similar or floccose, thinning out; teeth short, somewhat crowded, with pointed and fimbriate apex; hyphae 2.5–6 μ in diameter, with numerous clamp connections, loosely arranged next to the substratum and compact in the hymenium; cystidia 4–6 μ in diameter, little differentiated, axial or terminal, fascicled, projecting, septate and with clamp connections; basidia 18–35 $\times$ 5–7 μ, clavate, with 2–4 sterigmata; spores 7–10 $\times$ 3–4 μ, cylindrical, slightly curved, smooth, hyaline, granular or guttulate.

This species is recognized by its relatively large cylindrical spores and by the slender fascicles of projecting hyphae at the apices of the teeth. Bourdot and Galzin give the spore size as 7–8.5 $\times$ 2.75–3 μ. The measurement here given, however, is in accord with Bresadola's description. An authentic specimen at The New York Botanical Garden determined by Bresadola is like our Iowa specimens.

Two specimens were collected on much decayed oak wood near McGregor, Iowa, in August. This seems to be the first record of the occurrence of this species in North America.

10. ODONTIA SUDANS (Fries) Bres. Accad. Sci. Lett. Rovereto III. **3**: 100. 1897. (PLATE 2, FIG. 7.)

Hydnum Agardhii Fries, Syst. Myc. **1**: 418. 1821.

Hydnum sudans Alb. & Schw. ex Fries, Syst. Myc. **1**: 425. 1821.

Thelebolus sudans Fries, Elench. Fung. **2**: 51. 1828.

Grandinia Agardhii Fries, Epicr. 528. 1838.

Dacryobolus sudans Fries, Summa Veg. Scand. 404. 1849.

Porothelium Stevensoni Berk. & Br. Ann. Mag. Nat. Hist. V. 1: 23. 1878.

Porothelium confusum Berk. & Br. Ann. Mag. Nat. Hist. V. **1**: 24. 1878.

Grandinia exsudans Karst. Medd. Soc. Faun. Fl. Fenn. **9**: 51. 1882.

Grandinia sudans Lloyd, Myc. Notes **52**: 741. 1917.

Effused, membranaceous-ceraceous, separable in small pieces, warm buff to cinnamon-buff; margin similar, byssoid or pruinose, sometimes whitish; teeth short, scattered conical or short cylindrical, terminated by a viscid and more or less transparent, cylindrical or tapering, and projecting fascicle of cystidia; hyphae 1–3 μ in diameter, thin-walled, somewhat indistinct; cystidia 3.5–5 μ in diameter, walls thickened, septate, agglutinated and projecting as a prominent fascicle; basidia 15–24 $\times$ 3–4 μ, cylindrical-clavate, with 4 sterigmata; spores 5–7 $\times$ 1–1.5 μ, cylindrical, curved, smooth, hyaline.

The viscid bundles of cystidia are usually very conspicuous under a lens. Their contraction in drying and their more or less transparent appearance have been the cause of several striking errors in literature, as is evident by the rather extended synonymy. The teeth are often described as cupped or excavated, even by comparatively recent authors. This seems never to be true in fresh material. (Lohwag. Ann. Myc. 1931.) Fries in his Systema Mycologicum described the species under the name *Hydnum Agardhii* and apparently regarded *H. sudans* Alb. & Schw. as representing a doubtful species.

O. sudans is fairly common in Iowa. Collected from June to October on wood of deciduous and coniferous species. In Europe this species is reported on coniferous wood only. I have seen no report of its previous occurrence in the United States.

11. ODONTIA BARBA-JOVIS Fries, Epicr. 528. 1838. (PLATE 3, FIG. 1.)

Hydnum Barba-Jovis With. ex Fries, Syst. Myc. **1**: 421. 1821.

Hydnum Nyssa Berk. & Curt. Grevillea **1**: 100. 1873.

Kneiffia irpicoides Karst. Bidr. Finl. Nat. Folk **48**: 368. 1889.

Widely effused, soft floccose, loosely adnate, whitish to pinkish buff or slightly darker; teeth variable in size, not exceeding 4 mm. in length, soft, crowded, slender, blunt or subulate, fimbriate or terminated by one or more tapering tufts of cystidia, also bristly on the sides; hyphae 2–4 μ in diameter, thin-walled or thickened, with clamp connections; cystidia greatly elongated, 3–9 μ in diameter, cylindrical, thick-walled, becoming thin-walled and sometimes incrusted near the outer extremities, projecting in tufts at the apex of the teeth and singly on the sides; basidia 15–25 $\times$ 3–6 μ; spores 4–7 $\times$ 2.5–4.5 μ, oblong, smooth, hyaline, often 1-guttulate.

One specimen which I am referring tentatively to *Odontia Barba-jovis* is similar to *Odontia stipata* but differs in the more bristly teeth, the thick-walled, often capitate and more conspicuous cystidia and the smaller spores. The spore size is 4–5.5 $\times$ 2.25–3 μ, which is slightly less than reported by various European writers. This specimen differs also from material determined as *O. Barba-jovis* by Bresadola and Bourdot at The New York Botanical Garden in the smaller teeth and cystidia, some of which are incrusted at the outer extremities or bear a glistening and somewhat amorphous covering over the tips. Bourdot and Galzin (1927) describe a form with terminally incrusted cystidia. Two other Iowa specimens agree closely with the European conception of *Odontia Barba-jovis*. An authentic specimen of *Kneiffia irpicoides* Karsten and a fragment of the type of *Hydnum Nyssa* Berk. and Curt. at The New York Botanical Garden were also examined and seem clearly to be the same species.

Collected in October on deciduous wood near North Liberty, Iowa. Reported from several scattered localities in the eastern United States.

12. ODONTIA STIPATA (Fries) Quél. Fl. Myc. Fr. 435. 1888. (PLATE 3, FIG. 2.)

Hydnum stipatum Fries, Syst. Myc. **1**: 425. 1821.

Resupinate, effused, thin, soft floccose, loosely adnate, whitish becoming light buff; margin white, tomentose, sterile; teeth slender, entire or divided, pointed or sometimes slightly fimbriate, unequal in length; hyphae 2–3.5 μ, thin-walled to thick-walled, with clamp connections; cystidia 2.5–4 μ in diameter, emerging in terminal tufts, not strongly differentiated; basidia 12–20 $\times$ 3–5 μ, clavate to cylindrical, with 4 sterigmata; spores 4–6 $\times$ 3–4 μ, oblong, smooth, hyaline.

Odontia stipata is characterized by its very soft, floccose fructification, the pointed teeth and the emerging tufts of cystidia. The Iowa material closely resembles European specimens determined by Bourdot, Bresadola and Litschauer. It differs, however, from a waxy specimen in the Ellis collection at The New York Botanical Garden apparently determined by Bresadola, but this was clearly another species.

Collected once on decorticated elm log near Wellman, Iowa, in October. Reported from eastern United States. The Iowa specimen referred to *O. stipata* (Fries) Quélet by Cejp (1931) is *Radulum spathulatum* (Fries) Bres. as here understood.

13. (RADULUM ?) SPATHULATUM (Fries) Bres. Ann. Myc. 1: 89. 1903. (PLATE 3, FIG. 3.)

Hydnum spathulatum Schrad. ex Fries, Syst. Myc. 1: 423. 1821.

Irpex spathulatus Fries, Elench. Fung. 1: 146. 1828.

Effused, thin, soft, ceraceous or sometimes with a sub-ceraceous hymenial layer and floccose next to the substratum, often cracking in drying, cartridge buff to light ochraceous-buff; border similar or floccose and white; teeth 1.5 mm. or less in length, variable, subulate to cylindrical or spathulate to irpiciform, obtuse or pointed fimbriate or with pointed processes projecting from the sides and crests; hyphae 2–3 μ in diameter, with clamp connections, occasionally incrusted; cystidia not distinct, largely undifferentiated hyphae projecting from the hymenium or in bundles from the side and crest of the tooth; basidia $12–16 \times 3.5–5\ \mu$, clavate, with 2–4 sterigmata; spores $4–6 \times 2.5–4\ \mu$, subspherical to elliptical, smooth, hyaline, sometimes 1-guttulate.

This species is quite variable and lacks distinct diagnostic characters. It resembles *Odontia arguta* and is considered a form of this species by Miss Wakefield. However, the smoother and more compact, ceraceous fructification and the absence of the conspicuous, incrusted cystidia, seem clearly to separate the two forms. Litschauer referred several Iowa specimens of this species to *Radulum spathulatum* (Schrad.) Bres. He reports having had many specimens so determined for him by Bresadola and sent me a number of European specimens, including an authentic one from Bresadola. Whether Bresadola's conception is correct is not known. It does not seem to belong in the genus *Radulum*. The

frequently flattened and irregular teeth suggest characters of *Irpex,* but its general aspect and microscopic detail is too near *Odontia.*

Common in Iowa from early June to October, on wood of frondose and coniferous species. Collected throughout the year. Many specimens variously determined from the central and eastern United States were seen at The New York Botanical Garden and in other herbaria. A number of these were referred to *Hydnum pallidum* Cooke & Ellis.

14. ODONTIA ARGUTA (Fries) Quél. Fl. Myc. Fr. 435. 1888. (PLATE 3, FIG. 4.)

Hydnum argutum Fries, Syst. Myc. **1**: 424. 1821.

Odontia alutacea Bres. Atti Accad. Rovereto III. **3**: 97. 1897.

Effused, thin, soft, floccose, appearing pubescent, pruinose when dry, cartridge buff to cinnamon-buff; margin similar, thinning out or floccose; teeth 1–2 mm. in length, variable, at first short, then cylindrical or subulate, pointed, divided or penicillate at the apex, finely pubescent; hyphae 2–4 μ in diameter, distinct, with clamp connections; cystidia mostly 20–35 $\times$ 3–4 μ, fusiform or subulate, with incrusted terminations projecting from the hymenium, sometimes accompanied by others which are 4–6 μ in diameter, cylindrical, with obtuse or slightly enlarged, smooth or incrusted terminations, and up to 9 μ in diameter; basidia 10–16 $\times$ 4–5 μ, clavate, with 4 sterigmata; spores 5–6 $\times$ 4–5 μ, obovate, smooth, white, sometimes 1-guttulate.

This species resembles *Radulum spathulatum* (Fries) Bres. It can be distinguished readily by the variable but characteristic cystidia, the more floccose texture, and the pubescent appearance of the surface of the fructification. The hymenial cystidia with incrusted terminations separate it readily from other related species of *Odontia.* The fructifications are often so thin as to appear arachnoid under the lens.

O. arguta is abundant in Iowa on old wood of deciduous and coniferous species throughout the year. It was reported previously from Iowa by Cejp (1931) but this report is based on a species tentatively referred in this paper to *Radulum spathulatum* (Fries) Bres. Its distribution in North America cannot be determined from published notes. Dozens of herbarium specimens from many localities throughout central and eastern North Amer-

ica have been examined. Many of these were undetermined or referred to various names.

European specimens of *O. arguta* and descriptions by recent European writers indicate that our North American forms are identical. An authentic specimen of *Odontia alutacea* Bres. was examined at The New York Botanical Garden. *Hydnum caryophylleum* Berk. & Curt. seems to be a closely allied species.

15. ODONTIA BICOLOR (Fries) Bres. Ann. Myc. **1**: 87. 1903. (PLATE 3, FIG. 5.)

Hydnum bicolor Alb. & Schw. ex Fries, Syst. Myc. **1**: 417. 1821.

Hydnum subtile Fries, Syst. Myc. **1**: 425. 1821.

Odontia subtilis Quél. Fl. Myc. Fr. 435. 1888.

Hydnum serratum Peck, Ann. Rep. N. Y. State Mus. **50**: 112. 1897.

Hydnum echinosporum Vel. České houby. 745. 1922. (Fide Cejp.)

Widely effused, thin, adnate, soft, pruinose, becoming ceraceous in older portions, cracking slightly in the ceraceous portions, cartridge buff when fresh; margin pruinose, indeterminate, often quite wide, concolor or white; teeth up to 0.3 mm. in length, short, fragile, more or less regular in shape, obtuse or divided into several points, scattered evenly, more crowded in the older portions, crests when pointed usually composed of sterile hyphal ends; hyphae 2–3 μ in diameter, thin, collapsed and loosely agglutinated which somewhat obscures the hyphal characters, no clamp connections seen; cystidia 6–18 μ in diameter, terminating in a globose enlargement, covered with radiating crystals or sometimes containing a yellowish material, submerged or projecting; basidia 10–20 $\times$ 3–5 μ, clavate, with 4 sterigmata; spores 4.5–6 $\times$ 2.5–3 μ, oblong, obliquely attenuate, smooth, hyaline.

This species is easily recognized by its characteristic cystidia. The enlarged terminations of the smooth cystidia in many cases are collapsed and apparently empty. These differ from the cystidia in which the enlarged ends are covered with crystals by the fact that they are fewer in number or absent and arise from hyphae of greater diameter.

Collected once in Iowa on a prostrate poplar log in April. Specimens from New York, Michigan, Ohio, Louisiana, Florida,

Georgia and South Carolina also have been examined. These are identical with European specimens determined by Bourdot and by Litschauer. Morgan (1887) referred this species to *Hydnum nudum* Berk. & Curt. The specimens from Florida, Georgia and South Carolina were referred to *Grandinia granulosa* Fries. A fragment of an authentic specimen of *Hydnum subtile* Fries at The New York Botanical Garden and the type of *Hydnum serratum* Peck at Albany were studied.

16. ODONTIA CRUSTOSA (Fries) Quél. Fl. Myc. Fr. 436. 1888. (PLATE 3, FIG. 6.)

Hydnum crustosum Pers. ex Fries, Syst. Myc. 1: 419. 1821.
Grandinia crustosa Fries, Epicr. 528. 1838.

Fructification resupinate, effused, often orbicular, ceraceous-crustaceous, usually cracked when dry, pinkish buff to cinnamon-buff, sometimes more yellowish; margin pruinose or narrowly floccose, white; teeth small, subulate to short cylindrical, obtuse, sometimes weakly divided into several processes each of which is terminated by a slightly projecting bundle of sterile hyphae, scattered or crowded; hyphae 2–4 μ, with clamp connections, 3–5 μ at the apex of teeth; cystidia fusiform or subulate, few to numerous in the hymenium, barely emerging, of the same diameter as the basidia, thin-walled; basidia $15–30 \times 4–6\ \mu$, with 4 sterigmata; spores $6–8 \times 3–4\ \mu$, subcylindrical, smooth, hyaline, occasionally 1-guttulate.

The subulate, hymenial cystidia are differentiated chiefly by their shape and might well be regarded as paraphyses. This species may be separated from *Odontia crustula* by the subulate hymenial structures and the slightly larger spores. Two specimens at The New York Botanical Garden determined by Bresadola, represent the species as here understood. Three specimens determined by Litschauer have also been studied and appear to be identical. Several specimens of *Odontia crustosa* from Iowa were verified by Litschauer.

Common in Iowa on oak and other deciduous species; collected from April to December. Most of the many specimens from North America referred to this species at The New York Botanical Garden clearly represent other species.

17. **Odontia crustula** sp. nov. (*crustula,* a little crust). (PLATE 3, FIG. 7.)

Effusa, tenuis, crustaceo-ceracea, adnata, paulo secedens, cremea; verrucae breves, conicae vel breviter cylindraceae, obtusae, incisae, apicibus albidis; hyphae 2.5–6 μ, nodoso-septatae; cystidia 4–6 μ diam., cylindracea, fasciculata, adglutinata, incrustata, paulo exserta; sporae 4–6 $\times$ 3–3.5 μ, ellipsoideae, leves, hyalinae.

Widely effused, thin, crustaceous-ceraceous, adnate, slightly cracking, cream buff; margin indeterminate, pruinose, or minutely and narrowly floccose, sometimes slightly fimbriate and rhizomorphic, white; warts short, conical to short cylindrical, very obtuse, usually with the crests divided into whitish processes; hyphae 2.5–5 μ in diameter, thin-walled, clamp connections present, incrusted in the axial portion of the tooth; cystidia 4–6 μ in diameter, cylindrical, in heavily incrusted, more or less agglutinated bundles, projecting 15–100 μ at the apex of the tooth; basidia 15–25 $\times$ 4.5–7 μ, clavate; spores 4–6 $\times$ 3–3.5 μ, ellipsoid, smooth, hyaline or granular, sometimes guttulate.

This species is ceraceous and is characterized by one or more bundles of incrusted and relatively unspecialized cystidia at the crest of the teeth, as is common in many of the species of *Odontia.* However, I have been unable to refer it to any known species. Litschauer reports that he knows no European species to which it may be referred. *Odontia crustosa,* which externally resembles this species, has slightly larger spores and subulate, hymenial cystidia.

Fairly common in Iowa on decorticated wood and bark of deciduous and coniferous species. Collected from June to October. Type specimen collected near Milford, Iowa, on linden, June 16, 1931, by L. W. Miller, and deposited in the mycological herbarium of the State University of Iowa.

18. ODONTIA LIVIDA Bres. Nuovo Giorn. Bot. Ital. **23**: 158. 1891. (PLATE 3, FIG. 8.)

Resupinate, widely effused, ceraceous-crustaceous, cracked, typically honey-yellow but varying from chamois to clay color; margin not differentiated, or whitish, fimbriate; teeth deformed, short rigid, hispid at the apex; hyphae 2–6 μ in diameter, without clamp connections; cystidia 4–8 μ in diameter, cylindrical, elongated, usually heavily incrusted and occurring in compact, branching fascicles which project 25–250 μ at the apex of the teeth; basidia 18–32 $\times$

5–7 μ, elliptical, smooth, with granular content, sometimes 1-guttulate, faint yellow in mass.

The honey-yellow color, the rigid and hispid spines and the large spores are characters which distinguish this species. An authentic specimen from Bresadola at The New York Botanical Garden has been studied. It is quite like our Iowa specimens. The descriptions of *Odontia corrugata* (Fries) Bourd. & Galz. and *Odontia junquillea* Quél. indicate a very similar fungus.

On linden and oak wood from April to August; fairly common in Iowa. I have seen no previous record of its occurrence in the United States.

19. ODONTIA FUSCO-ATRA (Fries) Bres. Atti Accad. Rovereto III. 3: 95. 1897. (PLATE 3, FIG. 10.)

Hydnum fusco-atrum Fries, Syst. Myc. **1**: 416. 1821.

Hydnum carbonarium Peck, Ann. Rep. N. Y. State Mus. **40**: 55. 1887.

Odontia membranacea (Fries) Bres. Atti Accad. Rovereto III. 3: 95. 1897.

Acia fusco-atra (Fries) Pat. Tax. Hymén. 69. 1900.

Acia membranacea (Fries) Bourd. & Galz. Bull. Soc. Myc. Fr. **30**: 258. 1914.

Effused, soft ceraceous, adherent, color variable, dark-gray or fawn color to burnt umber when fresh, cinnamon-buff to fuscous-black in the herbarium; margin pruinose or radially fibrillose, usually lighter in color, sometimes not differentiated; teeth 0.5–2 mm. in length, stout, subulate, mostly entire and pointed or slightly divided at the crest, sometimes ciliate, often remaining pale at the apex; hyphae 3–4 μ in diameter, distinct, with clamp connections, parallel and compact in the teeth, incrusted singly or in fascicles in the axial portion, crystals minute; cystidia 4–5 μ in diameter, cylindrical, incrusted, elongated, arising from the axial portion of the teeth, sometimes a few inconspicuous, thin-walled, subulate, hymenial cystidia are also present; basidia 15–25 × 3–5 μ; spores 4.5–6 × 2–3 μ, short cylindrical, barely depressed on one side, smooth, hyaline.

O. fusco-atra resembles *O. uda* in structure but is readily separated by its dark color. The hyphae are generally larger, more distinct and with more numerous clamp connections. The incrusted hyphae and cystidia are less fascicled.

This species varies considerably in color. The lighter forms tend to have a more distinct margin and are colored slightly in KOH solution. *Hydnum carbonarium* Peck is based on such forms. A specimen of *Odontia membranacea* (Fries) from Bresadola and one from France (Galzin) at The New York Botanical Garden seem to be identical with light colored forms which I am here including in *O. fusco-atra.* Specimens of *Odontia fusco-atra* from Bourdot and from Bresadola were also studied. According to Bresadola *O. membranacea* differs from *O. fusco-atra* in the more slender and crowded teeth and in the slight difference in the color of the initial growth. These differences noted by Bresadola and observed in the specimens which I have studied seem to be due to variation only. The two forms merge and are identical in microscopic characters.

O. fusco-atra is fairly common in Iowa from March to late in November; collected on wood of various frondose species. Previously reported from scattered localities in the eastern United States.

20. ODONTIA UDA (Fries) Bres. Atti Accad. Rovereto III. 3: 97. 1897. (PLATE 3, FIG. 9.)

Hydnum udum Fries, Syst. Myc. **1**: 422. 1821.

Acia uda Bourd. & Galz. Bull. Soc. Myc. Fr. **30**: 255. 1914.

Effused, soft ceraceous, adherent, mustard yellow to chamois or tawny; margin radially fibrillose or pruinose, sometimes subhyaline; teeth usually crowded, slender, subulate, entire or fimbriate and divided at the crest, apex often white after drying; hyphae 2–4 μ, thin-walled, with rare clamp connections, compact, incrusted in the axial portion of the tooth; cystidia 2–5 μ in diameter, cylindrical, only slightly differentiated, usually emerging in incrusted fascicles; basidia 15–25 $\times$ 3.5–5 μ, clavate, with 4 sterigmata; spores 4.5–6 $\times$ 2–3.5 μ, ellipsoid, slightly depressed on one side, smooth, granular or hyaline.

The fructification turns purple upon contact with a KOH solution. This is often a useful taxonomic character. The fascicles of incrusted cystidia in the teeth usually stand out prominently upon adding KOH. Our specimens agree closely with European material determined by Bourdot, Bresadola and Litschauer.

Common in Iowa on wood of frondose species from March to October, and apparently occurring in the central and the eastern United States. This species was previously reported from Iowa by Cejp (1931).

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EXPLANATION OF PLATES

All figures drawn with the camera lucida. The longitudinal section of a tooth in figure 8, plate 3 at an initial magnification of 175 diameters, reduced to $\times$ 106 in reproduction; all others at an initial magnification of 1650 and reduced to $\times$ 1000.

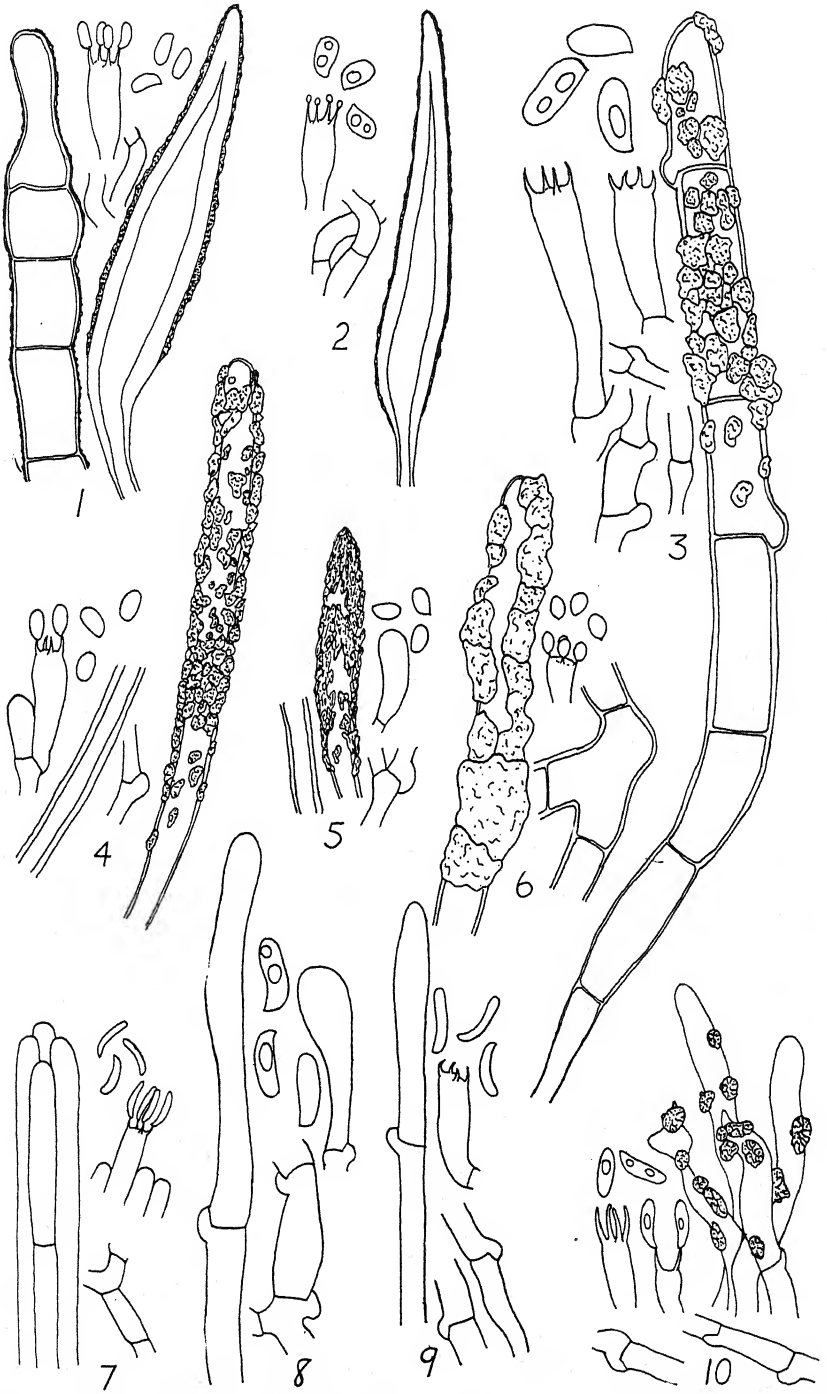
Cystidia or at least their terminal portion, hyphae, basidia and spores are shown in each figure.

PLATE 2

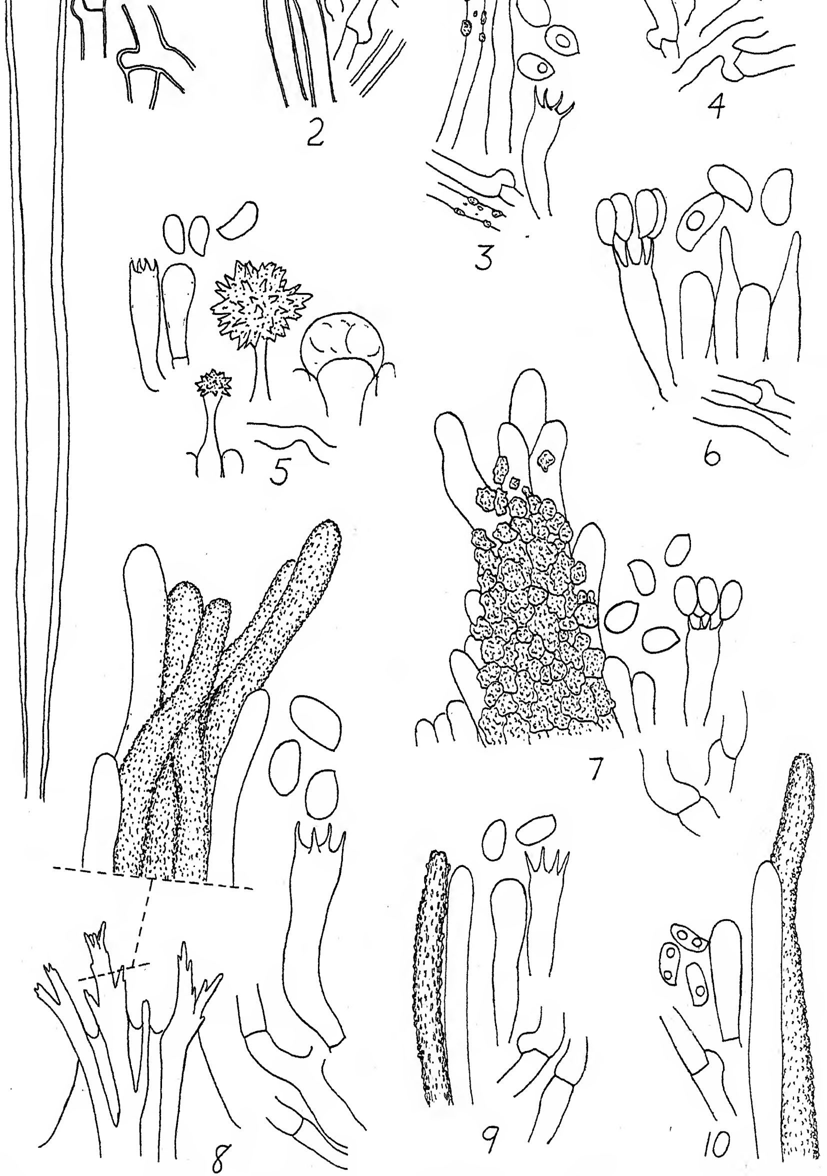
Fig. 1, *O. hydnoides;* 2, *O. Queletii;* 3, *O. setigera;* 4, *O. fimbriata;* 5, *O. ciliolata;* 6, *O. laxa;* 7, *O. sudans;* 8, *O. subalbicans;* 9, *O. alutacea;* 10, *O. cristulata.*

PLATE 3

Fig. 1, *O. barba-jovis;* 2, *O. stipata;* 3, (*Radulum ?*) *spathulatum;* 4, *O. arguta;* 5, *O. bicolor;* 6, *O. crustosa;* 7, *O. crustula;* 8, *O. livida;* 9, *O. uda;* 10, *O. fusco-atra.*



ODONTIA SPECIES



Odontia Species

3

A NEW SPECIES OF HELICOCEPHALUM

Charles Drechsler

(with plate 4)

The genus *Helicocephalum* was erected by Thaxter[1] in 1891 to make provision for a fungus that he found in a laboratory culture on carrion, and accordingly described under the appropriate binomial *H. sarcophilum*. In the brief discussion close resemblance to a large *Mortierella* or *Syncephalis* was pointed out, the similarity obviously applying more especially to the scattered distribution of the plant on the substratum, to the small diameter and aseptate or rarely septate condition of the vegetative hyphae, to the pronounced differentiation of the simple erect sporiferous hypha, and to the presence on the latter of rhizoid-like supporting basal attachments. Features distinguishing the fungus from any of the known genera of Mucoraceae were recognized in the character of the unusually large brown spores, as well as in their development through maturation of segments resulting from the insertion of septa at intervals in the helicoid distended terminal portion of the fertile hypha.

Although more than four decades have elapsed since the appearance of Thaxter's publication, the mycological literature of the intervening period would seem to offer no record of any encounter at first hand either with *Helicocephalum sarcophilum* or with any form sufficiently similar to be recognized as a congener. It may therefore not be unprofitable to set forth the main characteristics of such a congeneric form that made its appearance early in 1933 on rather old maize-meal agar plate cultures originally planted with decaying rootlets. On the mycelia of various species of *Pythium* that first extended themselves through the substratum had become superimposed a mixture of plant and animal life including an abundance of bacteria, nematodes of various species evidently feeding on the bacterial slime, several fungi preying on the nematodes,

[1] Thaxter, Roland. On certain new or peculiar North American Hyphomycetes. II. Bot. Gaz. **16**: 201–205. 1891.

amoebae of several types feeding on the bacteria as well as on the conidia of the nema-capturing fungi, and several species of minute phycomycetes preying on the smaller amoebae. The virtually complete degeneration of the *Pythium* mycelia had restored to the culture a degree of transparency little inferior to that of the medium originally; so that the delicate rangy hyphae of the fungus in question could be followed for long stretches with a water-immersion objective of high magnification. Optical conditions were therefore not unfavorable for uncovering possible mycelial relationships indicative of sarcophogy, opportunity for which the presence of fairly numerous dead nematodes and dead amoebae undergoing destruction by their fungous predators might be presumed to have supplied. However, even though occasionally somewhat denser branching could be made out in close proximity to a nematode freshly captured and killed, the visual evidence of sarcophagy was for the most part not especially striking. Yet the presumption for the fungus of a generally sarcophilous character comparable to that of Thaxter's species is perhaps not to be entirely dismissed, as some of the soluble substances in the dead microscopic animals could hardly have failed to diffuse into the agar substratum and thus become more widely available.

While in all phases of morphology and development the fungus under consideration shows close parallelism with *Helicocephalum sarcophilum,* its dimensions throughout are so much smaller as to leave no doubt that one is dealing with a distinct species. The vegetative hyphae are more delicate, measuring in diameter only slightly more than one-half of the 2.0 μ given by Thaxter. The fertile hypha which in Thaxter's fungus measures 1 mm. or more in height, here is usually only about one-half as tall. The basal diameter of the sporophore, which in the description of *H. sarcophilum* is given as 20 to 25 μ, is approximately only two-thirds as great in the present fungus, and an inferiority only slightly less pronounced is evident with respect to the apical diameter. In *H. sarcophilum* up to 21 spores are produced on one fertile hypha, whereas in the present form, rarely more than 10 are developed in a single head. Moreover the dimensions of the matured spores which in Thaxter's description are given as $55 \times 30\,\mu$ (maximum $65 \times 35\,\mu$) are represented in the present form by values nearly a

third less. As in spite of the smaller dimensions relative to *H. sarcophilum,* the new fungus is yet one of impressive proportions, a specific term having reference rather to the small number of spores is proposed as tolerably appropriate.

Helicocephalum oligosporum sp. nov.

Sparsum; hyphis sterilibus 1.0–1.3 μ crassis, fertilibus cylindraceis, sursum tenuatis, 0.35–0.6 mm. altis, 13–16 μ crassis, sursum 5.5–7.0 μ crassis; conidiis in spira 5–10, elliptico-cylindraceis, utrinque obtuse rotundatis, maturitate brunneis, 32–45 × 20–25 μ, demum secendibus et in capitulum subglobosum viscosum cohaerentibus.

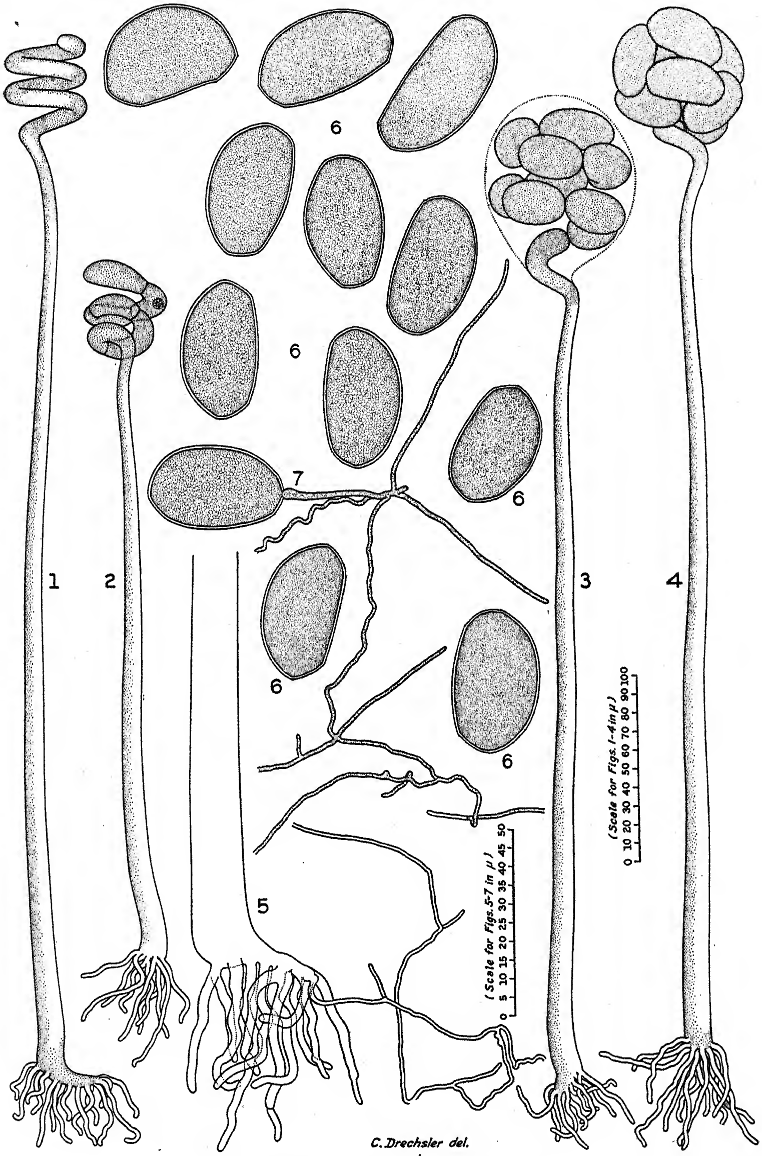
Sterile hyphae hyaline, creeping, rather sparsely branching, mostly 1.0 to 1.3 μ in diameter, devoid of septa except where living portions are contiguous to evacuated portions. Fertile hyphae sparsely scattered, hyaline, mostly 0.35 to 0.6 mm. in height, 13 to 16 μ in diameter at the base, where individually they are supported by rhizoids usually 10 to 20 in number and measuring 20 to 55 μ in length and 2 to 4 μ (average 2.7 μ) in diameter; tapering gradually upward to a diameter of 5.5 to 7.0 μ, then widening into a terminal portion abruptly coiled in usually 2 to 3 (mostly 2½) rather close helical dextrorse turns; the helicoid portion except for the proximal half turn, following insertion of septa at regular intervals, becoming converted at maturity into a chain of usually 5 to 10 spores. Spores brown, with finely granular contents, somewhat asymmetrically prolate ellipsoidal, obliquely truncate at either end (except terminal spore which is symmetrically rounded at distal end); provided with a wall mostly 0.5 to 1.0 μ in thickness; measuring 32 to 45 μ (average 38.7 μ) in length by 20 to 25 μ (average 22.5 μ) in diameter; separating and ultimately cohering in a rounded mass.

On laboratory culture prepared from decaying spinach (*Spinacia oleracea* Mill.) roots collected near Diamond Springs, Va., November 25, 1932.

Because of lack of evidence which might have referred *Helicocephalum sarcophilum* elsewhere, Thaxter prudently assigned the plant to the Hyphomycetes, adding, however, that it might eventually find a place among the Mucoraceae. In this connection the condition of the mycelium with respect to septation is deserving of some attention. The characterization of the sterile hyphae in the definition of the genus as "septate or rarely septate" would hardly encourage any very definite opinion concerning the taxo-

nomic affinities involved. Undoubtedly the difficulties attending the removal to microscopic mounts of vegetative mycelium from an opaque solid substratum in adequately extensive tracts must have been very considerable, and may have been only partly overcome. At any rate the rather meager representation of basal rhizoids in Plate XIX, Figure 1, accompanying the account suggests that a more than negligible degree of difficulty may have intervened even in the examination of these sturdier and more easily accessible structures. Such difficulty was fortunately not encountered in dealing with *H. oligosporum* in agar plate cultures, in which uninjured living vegetative filaments could be followed under high magnification to any lengths desired. The examinations thus facilitated revealed that whereas a septum was indeed present here and there, it always delimited a portion of filament filled with protoplasm from a contiguous portion of empty hyphal envelope. In no case were septa found inserted between adjacent masses of protoplasm within the vegetative thallus. In short the occurrence of septa was constantly associated with the evacuation or degeneration of protoplasmic contents in localized portions of originally continuous filaments, and was therefore thoroughly analogous to the occurrence of septa in the mycelium of the Phycomycetes generally.

The genus *Helicocephalum* may therefore with tolerable certainty be transferred to the Phycomycetes, apparently finding its most congenial place, as Thaxter well surmised, among the Mucoraceae. In this family, to be sure, it will occupy, hardly less than would have been the case in 1891, an isolated position; yet the isolation now becomes somewhat less conspicuous in view of the various other genera of curious morphology that have during the past four decades been definitely added here or are being assigned here with increasing confidence. As discovery of a sexual stage would be very useful in determining further the closer affinities of the genus, and since such a stage has not been found to occur hitherto in cultures of *H. oligosporum,* possibly because of a heterothallic condition, it is hoped that further strains of the same species may ultimately be found. Somewhat unfortunately the fungus has failed to grow in pure culture on any of the artificial media tried out so far, the spores failing to germinate and finally de-



HELICOCEPHALUM OLIGOSPORUM

generating when placed on sterilized agar substrata, though germinating fairly readily in the presence of contaminating bacteria and protozoa. For its conservation in living condition tube cultures representative of the same sort of biological mixture as that in which it originally made its appearance, have been successfully employed.

U. S. DEPARTMENT OF AGRICULTURE,
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EXPLANATION OF PLATE 4

Helicocephalum oligosporum. All figures drawn with the aid of the camera lucida, the magnification of those bearing numerals from 5 to 7 being exactly twice that of those numbered from 1 to 4. Fig. 1, Fertile hypha of approximately average definitive dimensions, showing basal rhizoids and terminal helicoid portion; $\times$ 250; 2, Fertile hypha of relatively small stature, after appearance of septa in the helicoid portion; $\times$ 250; 3, Fertile hypha after maturation of the brown spores, the original spiral arrangement of which is still maintained within the drop of extruded watery liquid; $\times$ 250; 4, Fertile hypha with ripe spores now disconnected but still cohering in a dry mass; $\times$ 250; 5, Basal portion of fertile hypha, showing connection with the mycelium from which it had origin, and relation to basal rhizoids; $\times$ 500; 6, Mature spores showing profile of each as viewed from along the axis of the spiral structure from which it originated; $\times$ 500; 7, Mature spore germinating with germ tube arising from truncate end; $\times$ 500.

ARTIFICIAL MANURE FOR MUSHROOM PRODUCTION [1]

Selman A. Waksman and C. A. Reneger

(with text figure)

For the commercial production of mushrooms, horse manure, properly composted, was found to be, up until the present time, the only important substrate. All attempts to develop a compost from plant residues and inorganic fertilizer, without the aid of the digestive system of the horse, have so far given rather unsatisfactory commercial results, although the feasibleness of such a process has been definitely established (1–4).

Horse manure contains about 70 to 80 per cent moisture and about 2 per cent of nitrogen, on a dry basis; a part of this nitrogen is in a water soluble form, largely as urea and ammonia, and a part in an insoluble form, largely as protein. When horse manure is placed in composts and allowed to undergo aërobic decomposition, four distinct processes are found to take place (5, 7): (1) a gradual decomposition of the carbohydrates comprising the two major groups of these complexes present in the manure, namely, the cellulose and hemicelluloses; (2) a rapid transformation of the water soluble forms of nitrogen in the manure into insoluble organic nitrogenous compounds, accompanied by an increase in the relative total nitrogen content, because of the reduction of the total dry matter in the manure; (3) an increase in the relative content of the lignin and its derivatives, which resist rapid decomposition by the microörganisms active in the compost; (4) an increase in the relative ash content of the compost, parallel to the reduction of total dry matter, due to the accumulation of the mineral constituents in the process of decomposition; if no other data are available, one can measure the loss of total organic matter as a result of decomposition by the increase in ash concentration. When the total quantity of material in the compost has been re-

[1] Journal Series paper, New Jersey Agricultural Experiment Station, Department of Soil Microbiology.

duced to about 50 to 70 per cent of the original weight, as determined on a dry basis, the carbohydrates will be found to have been reduced to a considerably greater extent than the total material, the lignins to a less extent, while the mineral constituents will have increased in proportion to the disappearance of the organic substances; the protein content of the compost will have increased even more markedly, due to the synthesis of microbial cell substance by the microörganisms active in the decomposition of the various constituents of the manure (**5–8**). This is brought out in table 1.

TABLE 1

COMPARATIVE CHEMICAL COMPOSITION OF FRESH AND COMPOSTED HORSE MANURE[1]

Chemical Constituents	Per Cent of Total Dry Material		Per Cent of Ash-free Material	
	Fresh Manure	Composted Manure	Fresh Manure	Composted Manure
Fats and waxes	1.47	0.95	1.93	1.83
Cold water soluble organic matter	3.02	1.59	4.00	3.06
Hot water soluble organic matter	2.73	1.51	3.59	2.90
Hemicelluloses	11.28	5.79	14.84	11.13
Cellulose	25.05	12.59	32.96	24.21
Lignin	21.59	15.44	28.41	29.70
Total nitrogen	1.29	1.57	1.70	3.02
Water insoluble protein	5.94	8.56	7.82	16.46
Ash	24.1	47.8	—	—

[1] The fresh manure has been kept in a heap for several months and has already undergone decomposition, as compared with manure recently collected.

Horse manure, consisting of bedding (straw), droppings and urine, is a fairly well balanced medium for decomposition to proceed rapidly, so that it does not require any added material to result in a good compost; it must only be kept moist and aërated occasionally, when the temperature of the compost becomes too high. However, frequently even horse manure must receive some added material in order to result in a good compost: when the manure has been collected from stables which have been kept particularly clean, thereby not receiving a large part of the urine, the addition of a small amount of inorganic nitrogen salt will be

found to hasten the process of composting and will result in a much better compost; when the manure consists largely of droppings with too little bedding, the addition of some straw will be found to be very helpful in giving to the compost a better physical condition and in resulting in a better and more abundant substrate for the mushroom fungus.

With these elementary principles in mind, it is easy enough to proceed with the preparation from horse manure of composts for the growth of mushrooms. One has merely to follow well established practices. However, the problem becomes complicated when one attempts to produce composts only from plant residues. It becomes then essential to know the chemical composition of the plant material that is to be used, as well as to determine the nature and rate of its decomposition under different conditions.

In the preparation of composts from such plant residues as straw, which are poor in nitrogen and in the essential minerals, namely, phosphorus, potassium and calcium, inorganic salts have to be added to the compost, in order to enable the fungi, bacteria and other microörganisms which bring about the decomposition of the plant material to develop rapidly and hasten the process of composting. These microörganisms use the straw constituents, especially the carbohydrates, as sources of energy and the nitrogenous and mineral constituents for nutritive purposes, thereby transforming the latter into microbial cell substance. This can be most easily demonstrated by the transformation of the inorganic nitrogen compounds added to the compost into organic forms, namely, constituents of the microbial cells.

Lambert (4) found that in the preparation of artificial composts from straw, the temperature is never as high as in composts of horse manure, the latter usually reaching 65–72° C., while in the former 49° C. was the highest temperature attained. The reaction of the compost, as expressed by the pH value, is less uniform and the buffer content is lower in the artificial composts; this points to an insufficient decomposition, which may be responsible for the low yields of mushrooms obtained. Hein (1, 2) also reported poor yields from composts prepared from straw and inorganic fertilizer. An attempt was later made to use soy-bean stover and mixtures of stover and straw. Here, as well, the tem-

perature never reached higher than 49° C., except when horse manure was added. A period of 12 weeks was required for composting and the results obtained were no more encouraging than those with the straw alone.

In preparing composts from straw as the only plant residue, to which inorganic salts are added, several difficulties are encountered, chief among which is the slowness with which the straw becomes thoroughly wetted and the delayed attack of the straw by the microörganisms. Cereal straw in a mature state does not represent a very ideal physical and chemical medium for the activities of microörganisms. If one were to use, however, leguminous plants and other green materials high in water-soluble substances and in nitrogen, and low in cellulose and in lignin, for the preparation of composts, a soggy mass is obtained which represents a highly unfavorable medium for the growth of the mushroom mycelium.

As a result of numerous studies on the composting of various plant materials, alone and in combination, it was found that a certain balance between cereal straw and a plant material in a green state can form an ideal mixture for the preparation of a mushroom compost. The green material, whether freshly harvested or allowed to dry, will hold the water and absorb the added water readily and will begin to undergo immediate decomposition by microörganisms. The temperature rises rapidly and within a few days the compost is ready to be turned over. At that time a uniform composting mass is obtained. The green material will supply the microörganisms with some of the nitrogen and the minerals which are required for the decomposition of the straw, although additional inorganic salts will be required, the amount depending on the nature of the materials used and their relative concentration. This can be illustrated by presenting the results of a typical experiment carried out on a small scale in New Brunswick.

Four composts were prepared as follows: 1. horse manure, consisting of droppings and bedding, freshly collected from the local stable; 2. a mixture of wheat straw (60 per cent) and of tobacco stems (40 per cent); 3. 60 per cent straw and 40 per cent dry alfalfa hay; 4. 70 per cent straw and 30 per cent tobacco stems.

The composts of plant residues received also ammonium phosphate (16 per cent nitrogen), at the rate of 5 parts of the salt for 100 parts of the straw used in the composts. The composts were properly watered and allowed to decompose for 44 days, turning them at frequent intervals. The temperature changes in the four composts are shown in figure 1. Although before the first turning

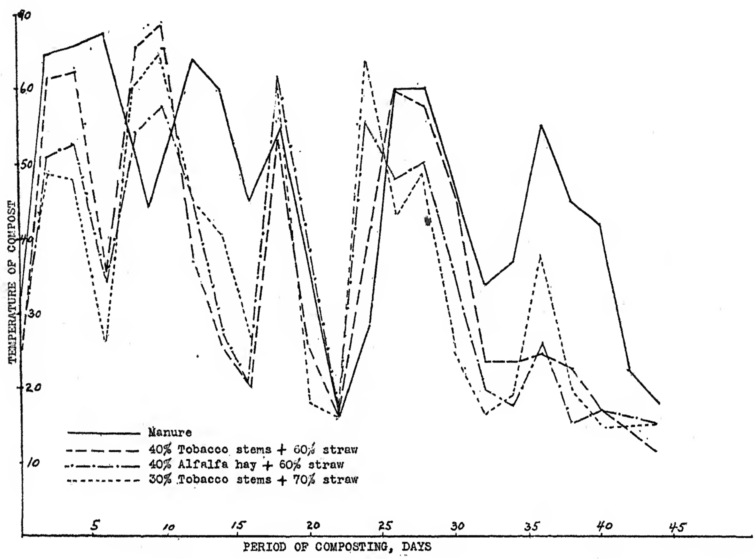

FIG. 1

the composts of the plant residues did not reach as high a temperature as that of the horse manure, after the first turning, the temperature of the former exceeded in all cases that of the latter.

The total dry weights of the composts at the beginning and at the end of composting are given in table 2, while the chemical composition of the dry material in the various composts is reported in table 3. The results show that the greatest loss of the organic matter took place in the compost consisting of straw and alfalfa, while the smallest loss occurred in the mixture containing 30 per cent tobacco stems and 70 per cent straw. As a result of the decomposition, there was a decrease in the water-soluble substances, in the fats and waxes and in the carbohydrates, accompanied by an increase in the mineral, protein and lignin constitu-

TABLE 2

DRY WEIGHT OF COMPOSTS AT BEGINNING AND END OF COMPOSTING

Number of Compost	1	2	3	4
Nature of Material	Manure	Straw (60%) + Tobacco Stems (40%)	Straw (60%) + Alfalfa Hay (40%)	Straw (70%) + Tobacco Stems (30%)
Total dry weight of material at start, lbs.	296.07	252.77	183.6	188.08
Total dry weight of compost, lbs.	182.20	138.04	67.34	124.21
Loss in weight, lbs.	113.87	114.73	116.26	63.87
Loss in weight, per cent	38.37	45.39	63.17	33.96

TABLE 3

CHEMICAL COMPOSITION OF COMPOSTS AT BEGINNING AND END OF COMPOSTING, PER CENT BASIS

Number of Compost	1 [2]		2		3		4	
	Start	End	Start	End	Start	End	Start	End
Moisture [1]	72.3	69.0	8.5	68.1	10.9	71.2	9.8	73.7
Total nitrogen in dry material	1.63	2.79	1.06	1.87	1.63	2.56	1.08	1.59
Total ash in dry material	15.74	24.97	19.72	32.14	8.68	13.35	15.81	18.75
Fats and waxes	2.24	0.74	1.57	0.92	1.88	1.17	1.55	1.52
Cold water soluble organic matter	4.93	2.63	9.96	3.49	8.77	4.47	8.13	3.27
Cold water soluble nitrogen	0.47	0.33	0.23	0.23	0.36	0.59	0.21	0.20
Total carbohydrates	39.35	24.83	40.26	30.85	51.73	45.00	44.90	42.41
Lignin	17.61	21.29	9.85	15.91	11.98	18.27	10.60	17.90
Crude protein	5.79	12.67	1.38	8.97	4.41	10.06	1.22	7.37

[1] In the case of the composts of plant residues, the moisture of the original material is given.

[2] See Table 2.

ents. The composts of the plant residues behaved as a whole in a manner similar to that of the horse manure, although some of the chemical constituents were transformed somewhat differently from a quantitative standpoint.

At the end of the decomposition period (44 days), the composts were transferred to regular mushroom beds, kept in a small, well ventilated dark room in the cellar of the building. The temperature of the room was then raised to 61° C. and the room thoroughly fumigated. A week later, the beds were spawned and

after the spawn was allowed to run for 28 days, the beds were cased with a light loam soil, to which 5 per cent of lime was added. The reaction of the composts was found to be about pH 8.0 in the case of the manure, somewhat more alkaline in the composts of straw and tobacco stems, especially where 40 per cent of the latter was used, and more acid in the case of the alfalfa-straw compost.

The spawn grew best on the alfalfa-straw composts, second best on the straw-tobacco stem composts and only third on the horse manure compost. The crop yields of the beds containing the various composts are given in table 4. The mushrooms began to

TABLE 4

YIELDS OF MUSHROOMS FROM COMPOSTS OF HORSE MANURE AND DIFFERENT PLANT RESIDUES

Average yield of bed, 8.16 square feet

Nature of Compost	Horse Manure	Straw (60%) + Alfalfa (40%)	Straw (70%) + Tobacco Stems (30%)	Straw (60%) + Tobacco Stems (40%)
Total yield, *grams* ..	3,798	2,550	3,989	416
Pounds per 1 square foot of bed space..	1.03	0.69	1.08	0.11

come first on the alfalfa-straw compost, next on the horse manure compost and only last on the straw-tobacco stems compost; the higher concentration of tobacco stems proved to be injurious to the production of the mushrooms, although not to the development of the mycelium. Whether this is due to the higher alkalinity of the composts or to the presence of a substance which in too high concentrations prevents the development of the mushrooms still remains to be determined.

The results show definitely that artificial composts can be prepared which are as satisfactory for the growth of mushrooms as horse manure. The greater degree of cleanliness, the better means of controlling the available material and the greater possibility for further improvement, all point to the probability of these composts taking in time the place of horse manure. However, the use of artificial composts will involve a greater knowledge of the chemical composition of the plant materials, of the processes of

decomposition of the plant residues in the compost and of the nutrition of the cultivated mushroom.

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THE SEXUAL MECHANISM OF SCLEROTINIA GLADIOLI [1]

F. L. Drayton

(WITH PLATES 5–7 AND 4 TEXT FIGURES)

Intensive cytological work has been done by several investigators on a few Ascomycetes, but in the majority of the genera there is no knowledge of the sexual mechanism involved in the formation of perithecia and apothecia. As a result, there is a dearth of fundamental information which would form the basis for any theories as to the phylogenetic relationships of this group and for a more thorough knowledge of the life histories of these fungi.

Cytological and experimental work with *Sclerotinia Gladioli* has revealed the presence of sex organs consisting of receptive bodies and microconidia; spermatization between compatible isolates being necessary for the production of apothecia. On the basis of this, in a paper to be published in Phytopathology, the writer proposes the new binomial *Sclerotinia Gladioli* (Massey) Drayton for the fungus previously known as *Sclerotium Gladioli* Massey, and includes a technical description of the fungus with illustrations of the sexual structures, and a brief historical resumé of the work on the fungus and the disease for which it is responsible.

It is only recently that the work on this organism has brought out these facts. In a preliminary account (**10**), the writer reported that the microconidia of *S. Gladioli* function as spermatia, and made reference to the structures in certain discomycetous lichens and in some of the Laboulbeniales, which are regarded as functional spermatia. Microconidia of the type found in *S. Gladioli* have been known for the past century and have been

[1] Contribution No. 124 from the Laboratories of Cryptogamic Botany, Harvard University.

Part of the work recorded here is taken from a thesis submitted to the Graduate School at Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy and the rest was done during the tenure of a National Research Fellowship in the Biological Sciences at the Laboratories of Cryptogamic Botany, Harvard University.

observed in species of the genera *Sclerotinia, Botrytis,* and *Sclerotium.* Their function has been a matter of much speculation, some authors considering them as functionless male cells, while de Bary (2), Brierley (4), and others have regarded them as true spores. We must pay tribute to the extraordinary insight of Tulasne who in 1863 described these structures (**15**), designating them as spermatia or conidiola, having prophesied in 1861 that, ". . . sometime or other it would be demonstrated that there resided in them a certain force or nature like that of pollen" (**14**). After Craigie (**7**) had demonstrated the sexual rôle of the pycniospores of the rusts, Whetzel (**16**) advanced the theory that these microconidia probably function as do the spermatia of the rusts. This has proved to be the case, for not only do the microconidia function as spermatia, but *S. Gladioli* and the rusts possess certain sexual phenomena in common. Later, Ames (**1**) working with *Pleurage anserina* (Ces.) Kuntze, has demonstrated the existence of sexual and genetical reactions in this fungus, which are similar in every respect to those found in *S. Gladioli.* As this situation was hitherto unknown in *Sclerotinia,* and involves indeed a new conception of sex in fungi, the results are presented here in detail.

EXPERIMENTAL DEVELOPMENT OF APOTHECIA

This fungus is known to develop mycelium, small resistant sclerotia, and microconidia. The sclerotia are minute structures, averaging $191 \times 164\,\mu$ in size, produced on the basal portion of the leaf sheaths and on the corm scales of diseased plants, and in and on all of the common culture media. Previous efforts to obtain sexually produced fruiting bodies were directed to the sclerotia, on the assumption that the fruiting bodies would be minute apothecia; but no such structures were produced in any of the trials.

Attention was then concentrated on a stromatic tissue as a possible source of fruiting bodies. Cultures on agar media develop a black tissue on their surface, but on sterilized wheat and water, this tissue is recognizable as a definite stroma, varying in thickness from 80–500 μ, and of different structure from the sclerotia. The latter have a compact rind and pseudoparenchymatous medulla,

while the stroma is prosenchymatous throughout, with a rind of loosely aggregated, black, septate, thick-walled, short hyphae (PLATE 7 E).

In the experiments with this stromatic tissue, portions of wheat cultures including the tissue were placed on moist sand and kept at 15° C. Some two weeks later, small, columnar, light brown, pilose structures, which were not apothecial fundaments, had developed from the stroma. With the hope that they might be receptive bodies awaiting fertilization by the microconidia, the following experiment was made. In four of these cultures, microconidial sporodochia from different isolates of the fungus were placed on the pilose structures with a needle. After 10 days, two lots showed definite development of elongated bodies easily recognizable as apothecial fundaments. The pilose structures had elongated and become branched, with less marked pilosity, darker color, and a depression at each tip; in this way resembling similar structures in the genus *Sclerotinia*.

When environmental conditions necessary for the development of apothecia had been determined, no difficulty was experienced in obtaining mature apothecia with asci and ascospores typical of those found in the genus *Sclerotinia*. The structure of this fruiting body is recorded in the separate paper to be published in Phytopathology. When the ascospores were discharged on potato-dextrose agar, they germinated in 8–10 hours and the resulting growth was identical with that of the original cultures.

Technique

The preliminary experiment provided some suggestion of the spermatial function of the microconidia and the receptive character of the pilose structures formed from the stroma. As indicated above, the confirmation of these results required considerable modification of the technique. This entailed primarily the determination of favorable cultural conditions for the development of microconidia and receptive bodies, and the procedures ultimately adopted for these phases of the work are described separately in the two succeeding sections of this paper. In addition, it was necessary to devise special methods for spermatization and for development of the apothecia, and these will be described here.

After several trials, a uniformly successful method for the process of spermatization was found. This involves a supply of suitable material, the preparation of a favorable soil extract, the application of a microconidial suspension to receptive bodies in the proper stage of development, and the maintenance of these at a suitable temperature. In addition, it was necessary to subject the fertilized cultures to appropriate conditions of light and temperature.

Material ideal for spermatization, was provided by wheat cultures in Petri dishes kept at room temperature for 17 days, followed by a temperature of 15° C. for 6–8 days. The contents of these Petri dishes were removed with a flamed scalpel and placed on a layer of sterilized, moist, sandy soil or sawdust, about ¾ inch deep in sterilized, 100 mm. preparation dishes. As a medium for making a suspension of the microconidia, a soil extract was prepared by intermittently shaking a mixture of 50 grams of soil and 500 cc. of distilled water for about an hour, filtering twice through filter paper, tubing, and then sterilizing for 30 minutes at 15 pounds pressure. By this procedure the soil extract was freed from any traces of copper in the distilled water, yielding a medium of an osmotic concentration comparable to that of the soil water with which the microconidia would be in contact in nature. A few drops of the soil extract were placed in a flamed watch glass, and into this, under the low power of a dissecting microscope, the microconidial sporodochia from the *Lycium*-potato-dextrose agar dishes were introduced with a flamed needle. The sporodochia were gently crushed with a flamed arrow-headed needle, so as to free the microconidia from their mucilaginous matrix. The suspension was then diluted with sufficient soil extract to obtain the amount required for spermatization; usually 8–10 drops of the suspension being sufficient for each Petri dish culture. Camel's hair brushes, sterilized by suspending them in boiling water for 15–20 minutes, were used for spreading the suspension over the receptive bodies, the brush being dipped repeatedly into the watch glass until the suspension was used up, if only one culture was to be spermatized. If, however, several cultures were to be spermatized, the suspension was dropped on the surface of each culture with a sterilized pipette and a separate

brush used to spread the drops over the receptive bodies. This precaution is necessary, because the wheat cultures often bear microconidia which would be introduced into the next culture spermatized. A layer of moist sandy soil or sawdust was then evenly distributed over the surface of the spermatized cultures, and a small quantity of plain soil extract added, so as to keep the upper and lower layers of soil or sawdust well moistened although not wet. The dishes were then placed in a 15° C. chamber.

Two kinds of test cultures were used in these experiments, in one of them, in addition to the spermatized plates, a second series of plates was subjected to similar treatment except that soil extract lacking microconidia was applied to the receptive bodies. In the other, to control the conditions still more exactly, and to make use of comparable portions of the same thallus, the cultures before being removed from the Petri dishes were cut in half, and the two halves spaced an inch or so apart on soil in a Stender dish. One half was then spermatized and the other half treated similarly with plain soil extract (PLATE 6*A*).

Further treatment of the cultures in which fertilization had been accomplished was as follows. After the apothecial fundaments became evident, usually in 8–10 days, they were kept under observation for another 4–6 days, when the depression at their apices was well defined. At this critical stage, they were removed to the light at a temperature maintained as near 15° C. as possible, direct sunlight being avoided by using a cheesecloth covering about 2 feet above the cultures. During the winter and early spring the greenhouse was satisfactory for this purpose, the apothecia expanded and matured in 12–18 days, depending on the amount of light prevailing, but as the season progressed it became necessary to use artificial light in a controlled cold chamber. Here the light was supplied by 60 watt, frosted, electric bulbs in gooseneck lamps, so placed that the bulbs were two feet above the covers of the dishes.

Experimental proof

Using the technique described above, extensive experiments were conducted with ten isolations of the fungus, which had been collected during the preceding years from various localities and

suscepts. The original designations of these cultures are here listed with brief descriptions.

G5—From a corm of one of the large flowering gladiolus varieties in a shipment from Holland, 1926.

SG2, SG3, SG4—From corms of large flowering and primulinus hybrid varieties of gladiolus grown in New York State, 1929–1931.

Gnl—From a corm of one of the small flowering or Colvillei type of gladiolus in a shipment from Holland, 1926.

C2—From a crocus corm collected in Holland in 1928.

Fla—From a freesia corm in a shipment from Southern Europe, 1927.

Flb, F2—From freesia plants collected in Long Island, N. Y., in 1929 and 1932 respectively.

S360—From gladiolus corms collected in Indiana in 1926.

Monomycelial transfers were made from these cultures by means of single hyphal tip isolations, and their specific identity was established by the uniformity of their growth and size of sclerotia on various culture media, and by successful inoculation in and on young gladiolus corms.

Each of the ten cultures is capable of producing both receptive bodies and microconidial sporodochia, which makes it possible to carry out reciprocal crosses between all of the isolates. In conjunction with adequate test cultures, this was done, and in the accompanying diagram, those crosses which resulted in apothecia are indicated by the symbol +, and those in which no fertilization occurred, by the symbol —.

Figure 1. It is evident that the receptive bodies of each isolate cannot be fertilized by microconidia from the same thallus, that is, they are self-sterile. In addition, three of the isolates are compatible with the other seven. Two groups are therefore represented in these ten isolates, one comprising C2, F2, and S360, and the other G5, SG2, SG3, SG4, Gnl, Fla, and Flb. These groups exhibit reciprocal inter-group fertility, reciprocal intra-group sterility, and self-sterility in every isolate.

Single and multiple ascospore cultures were made from discharged ascospores, 24 of the former and 5 of the latter, using apothecia from two different crosses. These cultures were planted

in duplicate on sterilized wheat in Petri dishes and when the receptive bodies appeared, one series was spermatized with microconidia from the isolate used as a source of receptive bodies in the original cross, and the other series was spermatized with microconidia from the isolate used as a source of microconidia in the

♂ \ ♀	G5	SG2	SG3	SG4	GN1	F1a	F1b	C2	F2	S360
G5	—	—	—	—	—	—	—	+	+	+
SG2	—	—	—	—	—	—	—	+	+	+
SG3	—	—	—	—	—	—	—	+	+	+
SG4	—	—	—	—	—	—	—	+	+	+
GN1	—	—	—	—	—	—	—	+	+	+
F1a	—	—	—	—	—	—	—	+	+	+
F1b	—	—	—	—	—	—	—	+	+	+
C2	+	+	+	+	+	+	+	—	—	—
F2	+	+	+	+	+	+	+	—	—	—
S360	+	+	+	+	+	+	+	—	—	—

Fig. 1. The result of reciprocal crosses between ten isolates of *S. Gladioli*. The symbol + indicates development of apothecia, the symbol — the absence of fertilization.

original cross. In other words, the receptive bodies developed from hybrid ascospores were back-crossed separately with each of the parent isolates. The results were highly significant, for the multiple ascospore cultures reacted with the microconidia of both parents, 12 of the single ascospore cultures reacted with the isolate used as a source of microconidia—the male parent, and the other

12 reacted with the female parent isolate. The number of cultures used is obviously too small to draw any definite conclusions as to the segregation ratio of the compatibility or sterility factor, but it is evident that segregation does take place and apparently in a 1 : 1 ratio.

In this and many other species of *Sclerotinia,* it has been observed that the ascospores, when placed in a comparatively non-nutritive medium such as soil extract or tap water, develop microconidia directly on the spores or on short germ tubes. These microconidia are capable of fertilizing receptive bodies, as shown by the following two experiments. The isolate C2 was planted on the cut surface of cooked gladiolus corms and in due time receptive bodies were produced in abundance. Two groups of these were spermatized with microconidia of the compatible isolate Gnl and in 10 days apothecial fundaments were well developed in the spermatized areas (PLATE 5 A), and these expanded into mature apothecia in two weeks and ascospores were discharged. Three weeks later, it was noticed that the surrounding receptive bodies, which had not been spermatized, were beginning to develop into apothecia (PLATE 5 B), which later matured, the original two groups of apothecia having dried up in the meantime. Under the moist conditions prevailing in these cultures, the ascospores which fell on the receptive bodies had presumably developed microconidia and some of these, approximately half of them, were capable of fertilizing the receptive bodies which had not been included in the two areas originally spermatized. To substantiate this assumption, six cultures bearing receptive bodies and consisting of isolates representative of both groups, were placed on moist sandy soil in preparation dishes, and four mature apothecia were attached to the lid of each dish so that the ascospores could be shot downwards to the receptive bodies. The apothecia used for this experiment were all the product of cross-spermatization between the isolates C2 and Gnl. In 10 days the six cultures were covered with apothecial fundaments, thus indicating that fertilization had been accomplished by the microconidia formed by the germinating ascospores. The segregation of the compatibility factor in the ascus therefore resulted in a condition whereby the microconidia produced by approximately half of the ascospores discharged by any

one apothecium were apparently compatible with the receptive bodies of any one isolate.

In order to prove that the development of apothecia was brought about by the fertilizing power of the microconidia rather than as the result of the stimulating action of some substance secreted by them, the following experiment was performed. A soil extract suspension of microconidia was made and half of it was filtered through a Berkefeld filter, which removed all of the microconidia. The filtered and unfiltered portions were then applied separately to four dishes with cultures bearing receptive bodies of two compatible isolates. Each portion was used on two cultures, one of one isolate and the second of the other. Optimum conditions of temperature and moisture were provided, and at the end of 14 days it was evident that the two dishes spermatized with the unfiltered suspension had developed apothecial fundaments, while a microscopic examination of the two cultures to which the filtered extract had been added, failed to show any development of the receptive bodies indicative of fertilization.

All possible vegetative pairings of the ten isolates were made to determine whether fertilization could be effected by the intermingling and fusion of hyphae of the thalli of any pair of isolates. These were kept under optimum conditions, but no apothecial fundaments appeared. On later flooding the preparations with water, and so washing a few microconidia from one thallus to the other in each plate, a few apothecia were produced where compatible pairs were present. None appeared where incompatible isolates were paired. It is obvious therefore that fertilization cannot be brought about as a result of hyphal fusion.

The microconidia of this fungus are therefore true spermatia or gametes, capable of fertilizing receptive structures formed on the stromata of certain other thalli with the consequent development of apothecia. The phenomenon of self-sterility and fertility only between certain isolates is significant, but not new among plants, for observations of this character have been made in several genera of hermaphroditic flowering plants.

The microconidia

Massey (13) first observed microconidial sporodochia in test tube cultures 25–40 days old, where they appeared as minute white granules buried in the medium and adjacent to the wall of the tubes. The writer made an effort to provide cultural conditions which would be more favorable for their production. Media composed of the stems of various plants combined with potato-dextrose agar were tried, and it was found that the stems of matrimony vine (*Lycium halimifolium*) used in this way in Petri dishes and kept at room temperature for 2–3 weeks provided the desired conditions. It is of interest to note that when sterilized soil is placed in a channel made by removing a strip of agar from the center of a Petri dish of solidified potato-dextrose agar, and the fungus planted on either side of the soil, microconidia develop in abundance on hyphae that penetrate the soil. Again, when sterilized soil is placed on two weeks old cultures of any favorable medium, sporodochia will develop in the soil. This is not a practicable means of obtaining microconidia for purposes of spermatization, but it indicates that these spores are probably formed in nature in the soil surrounding diseased plants.

Figure 2. The sporodochia appear at first as small milky droplets which later enlarge up to 1.5 mm. in diameter, become more opaque, and on drying become waxy in consistency. They consist of a central core of intertwined conidiophores arising from closely septate hyphae, which develop as branches from a main hypha running through the center of each sporodochium. Each conidiophore is verticillately branched and the ultimate branches are elongate, tapering cells which are curved and cut off microconidia exogenously from their apices in the form of loosely connected chains. A short isthmus is often evident connecting the spores with the terminal cells and this often remains attached to some of the spores. The microconidia are cut off in immense numbers and are embedded in a mucilaginous matrix which becomes waxy on drying, but is easily soluble in water. The microconidia are globose, 1.2 to 1.8 μ in diameter, hyaline, and contain a single nucleus which occupies about one third of the volume of the spore. When viewed in various positions of the spores the nucleus is seen to be cup-shaped (FIGURE 2).

Most investigators, including the writer, have failed to obtain any growth of the microconidia of this or other species of *Sclerotinia.* The few cases where successful germination is reported, remain to be satisfactorily explained.

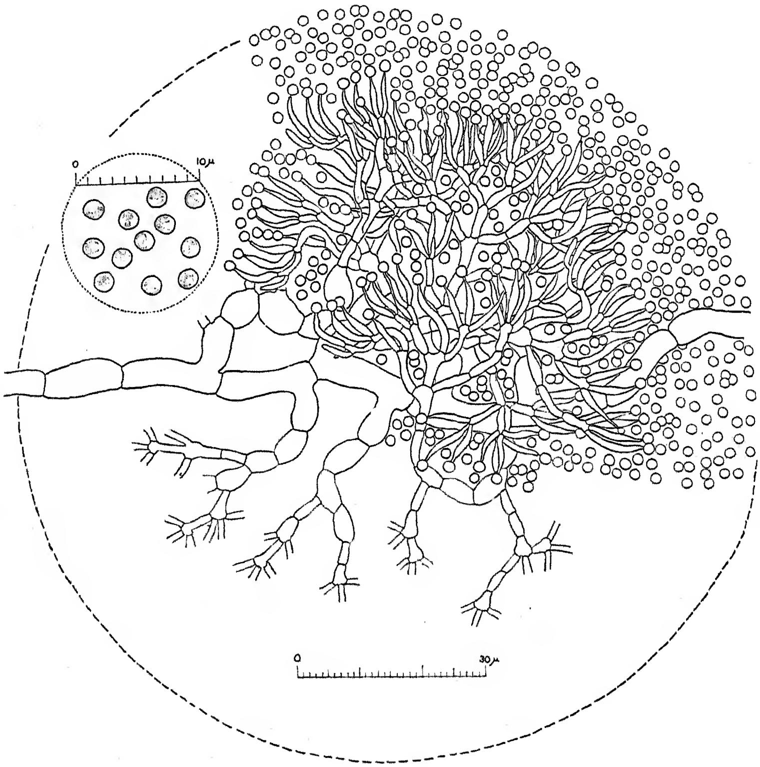

Fig. 2. A small microconidial sporodochium showing the conidiophores and the microconidia embedded in a mucilaginous matrix. Inset—some microconidia more highly magnified showing the shape and size of the nuclei.

The cultural conditions best suited to the production of receptive bodies are usually not favorable for the development of microconidia and vice versa. In a few of the matrimony vine and potato-dextrose agar plates, however, small stromatic areas were formed on portions of the stems close to the surface of the agar and these patches of stroma developed a few receptive bodies,

on which microconidial sporodochia later appeared (PLATE 6 B). This provides spectacular evidence of the monoecious or hermaphroditic character of a monomycelial thallus of this fungus, and, as these receptive bodies did not develop apothecia, the self-sterility of each isolate was again demonstrated.

THE STROMA AND RECEPTIVE BODIES

The stroma forms a discontinuous, partly separable layer on the surface of solid substrates rich in starch such as grains of wheat, rye, barley, etc., but on nutrient media like oatmeal, cornmeal, potato-dextrose agar, etc., the layer is a continuous and inseparable one. This difference is due to the uniform ease with which the hyphae can penetrate the latter media, while on grains of wheat only certain portions appear to be easily penetrable, notably the vicinity of the embryo. At these points the hyphae become established within the grains early in the development of the fungus, and later as the stroma develops it spreads over the rest of the grains in a separable and much thinner layer. The development of the receptive bodies takes place from these thicker stromatic areas, the amount of stored material there being obviously greater. In nature the stroma is only found in the corms of diseased plants, thus accounting for the necessity of a culture medium rich in starch for its development in the laboratory.

The structure of the stroma and its difference from that of the sclerotia has already been noted. The sclerotia apparently function solely as organs of resistance, while the stroma is the specialized tissue for the production of receptive bodies and apothecia. This is different from the situation in the species of *Sclerotinia* with true sclerotia so far studied, in which these two functions are combined in the one structure. This striking difference will be referred to again in a later section of this paper.

The receptive bodies are .8–1.9 mm. tall depending on their age, .4–.8 mm. broad, columnar, sometimes branched or cockscomb-shaped, tapering to a rounded apex or occasionally slightly capitate, light brown, pilose, and surrounded with a thin layer of mucilaginous substance. Externally, they possess a layer of loosely interwoven, thick-walled, septate hyphae, which are hyaline towards the apex, but become progressively darker towards the

base, merging with the black hyphae of the stromatic rind which may extend upwards to a distance of about one third the length of the receptive body. Within this are light brown, densely packed and intertwining longitudinal hyphae which at the apex give rise to a crown of thinner walled, septate, densely filled hyphae, which arch inwards to form a depression at the center of the apex (PLATE 7 A AND B.). In the center is a column of hyaline, less compact tissue tapering more or less sharply to the apex and consisting mainly of a sparsely septate, intricately coiled, multinucleate ascogonial system which terminates at the apex in trichogynous hyphae with their tips clustered beneath the overarching, sterile hyphae at the apex of the receptive body (PLATE 7 C AND D).

In order to determine the optimum conditions for the development of receptive bodies, the following experiment was carried on. The isolates G5, SG4, Gn1, C2, F1a, and F2 were grown at temperatures ranging from 12° to 30° C. at intervals of 3° and also at room temperature (18°–21° C.). The cultures used consisted of wheat grains and water in the proportions of 1 to 3 by weight, placed in Petri dishes, and sterilized at 15 lbs. steam pressure for 30 minutes. The plates were planted in a duplicate series and held at the various temperatures for 17 days, after which they were placed at 15° C. for 10 days, when notes were taken on the prevalence of the receptive bodies. The accompanying charts illustrate the results obtained.

Figure 3. It is evident that all of the isolates have their optima for receptive body formation within the range 18° to 24° C. Certain individual variations are exhibited, however; C2 and F1a are at the extremes as regards the readiness with which receptive bodies are produced. This variation is correlated with individual differences in rate of growth as determined by daily measurements of the thalli during the first 72 hours of their growth. Where no receptive bodies developed at 12° C. and at 30° C., there was no stroma present, and an increasing number of receptive bodies at the other temperatures was accompanied by increased amount of stroma.

Under these optimum conditions, the development of the receptive bodies is initiated by a protrusion on the surface of the stroma, which quickly elongates, leaving the black stromatic rind

towards the base of the receptive body. Prior to this, when the surface of the stroma is still flat, beneath the rind a clump of deeply staining, coiled hyphae may be seen, which have no connection with the surface. These hyphae apparently constitute the initials of the coiled ascogonial system (PLATE 7 E).

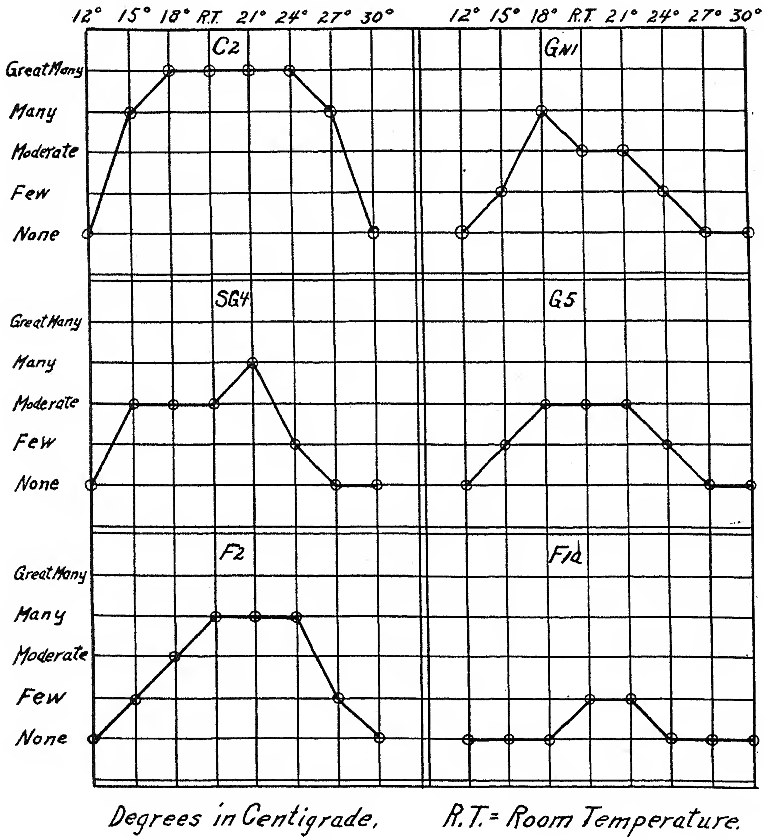

Fig. 3. Charts showing the relation of temperature during vegetative growth of the mycelium to receptive body production, in six isolates of *S. Gladioli.*

It was of importance to determine whether fertilization was restricted to the receptive bodies and the following experiment was devised with this end in view. A single receptive body was spermatized with a small amount of microconidial material from

a compatible isolate, and precautions were taken to prevent the spread of microconidia to neighboring receptive bodies. Some branching of this receptive body took place after spermatization, and each branch developed into an apothecial fundament. The surrounding receptive bodies had also branched and elongated slightly, but showed no evidences of fertilization. In this experiment, microconidia were also placed on stromatic areas from which receptive bodies had not yet developed, and there was no reaction of any kind. This proves that the receptive bodies alone possess the facilities for fertilization, the ascogonial coil being inaccessible prior to the development of the receptive bodies.

In another experiment with the same object in view, a series of 15 wheat cultures was planted with the isolate C2. Leaving plates 1, 7, and 15 unspermatized, the others were spermatized with microconidia from the compatible isolate Gn1, commencing with plate 2 on the fourth day after planting, and continuing at intervals varying from 1 to 3 days to plate 14, where spermatization was performed on the 23d day, when a considerable number of receptive bodies had appeared. All of the cultures except No. 14 and the checks were spermatized before any receptive bodies had formed. The whole series was then covered with moist sandy soil on the 23d day and put at 15° C. After two weeks, the first nine cultures in the series, spermatized between the 4th and 16th days showed a few apothecia developing in some, none in others. The 10th and 11th cultures, spermatized on the 18th and 19th days, just before the receptive bodies became plainly evident, had quite a large number of young apothecia. The 12th culture, however, spermatized on the 23d day when a great many receptive bodies were present, had an immense number of apothecial fundaments and the unspermatized ones had none. The fact that a few apothecia developed in certain cultures spermatized before receptive bodies appeared, indicates that some microconidia remained viable until receptive bodies developed. Fertilization could not have occurred through the stroma, for this possibility was eliminated by the experiment described above, and this experiment further excludes any possible interaction between microconidia and vegetative hyphae.

The development from receptive bodies to apothecial fundaments, or the failure of this to occur, is evident 10 days after spermatization. In the test cultures, in the self-spermatized cultures and in those spermatized with microconidia from an incompatible isolate, fertilization does not occur, as has been previously shown. This can be recognized macroscopically by the slight elongation of the receptive bodies, without change of appearance, and by the development of white patches of mycelium on the soil surrounding the receptive bodies; presumably a vegetative proliferation. The cultures in which crosses were made between compatible isolates soon show evidences of fertilization, by the absence of any mycelial patches and the apothecial fundaments are evident as short, black, erect columnar bodies, glistening with water, and with a slight depression at the apex (PLATE 5 A).

PRELIMINARY CYTOLOGICAL OBSERVATIONS

In view of the interesting results obtained in the experimental work on the sexual mechanism of *S. Gladioli,* some cytological studies were undertaken. No investigation of this kind has ever been made of any species of *Sclerotinia,* except that of Kharbush (12) who attempted to trace the nuclear evolution in the ascus of *Sclerotinia Fuckeliana* de Bary. In longitudinal sections of the apothecium he found binucleate cells in the ascigerous layer arising from two mycelial filaments which anastomosed at their apices and were separated from these branches by a septum. He traced the development of the asci from these cells and considered that the association of nuclei is accomplished at the point of anastomosis in a manner similar to that described by Dangeard (8) for *Peziza vesiculosa.* This author regards the situation in *Peziza* as analogous to that of *Eremascus* and *Dipodascus,* although he qualifies this statement by an observation that frequently the young ascus appears to have a different origin in which a "crozier" is involved. It is obvious that Kharbush's conception of the origin of the ascus is a misinterpretation, as he failed to recognize the sexual function of the microconidia, but his contribution lies in his description of the nuclear phenomena involved in the developing ascus, where he observed nuclear fusion to take place, and also described the subsequent divisions leading up to

the formation of the 8 ascospores. He is emphatic in his observations that the chromosome numbers of the fusion nucleus and nuclei after reduction division are four and two respectively.

In an attempt to trace the nuclear history of *S. Gladioli,* receptive bodies, apothecial fundaments, and apothecia were killed, imbedded in paraffin, and eventually stained in iron-alum haematoxylin and erythrosin. One series was spermatized with compatible microconidia, and then at daily intervals a number of receptive bodies were removed and killed; thus giving stages which were representative of the entire development of the fruiting body. Another series paralleled the first, but in this instance incompatible microconidia were used for spermatization. In both of the series some receptive bodies also were killed and fixed before the application of microconidia.

Many difficulties were encountered in devising a satisfactory technique for this study, and the complex structure of the receptive bodies tended to obscure many details of the nuclear history. In spite of these difficulties it seems desirable to record various points of interest that have come out of this preliminary cytological study.

Figure 4. The vegetative mycelium comprises cells containing usually 4 nuclei of approximately .5 μ in diameter. The microconidia borne on this mycelium have a single, cup-shaped nucleus that occupies about one third of the volume of the spores (FIGURE 2). The sparsely septate, coiled ascogonial hyphae show great variability in size, shape, number, and distribution in different receptive bodies. Similarly their many nuclei differ in size and number in the various cells of these hyphae (FIGURE 4 A). Considerable anastomosis takes place between the cells of the individual coils. The coils in turn give rise to elongate, almost straight hyphae which by the fact that they are less densely stained and of greater diameter, may be distinguished from the sterile apical hyphae that arch above them. These straight elongate hyphae are trichogynes because they disintegrate after spermatization with compatible microconidia, while at the same time the ascogonial coil exhibits decided modification, as will be described.

The microconidia, when placed on the receptive bodies are either drawn by capillary action into the channel formed by the apical hyphae and so brought into contact with the trichogynes,

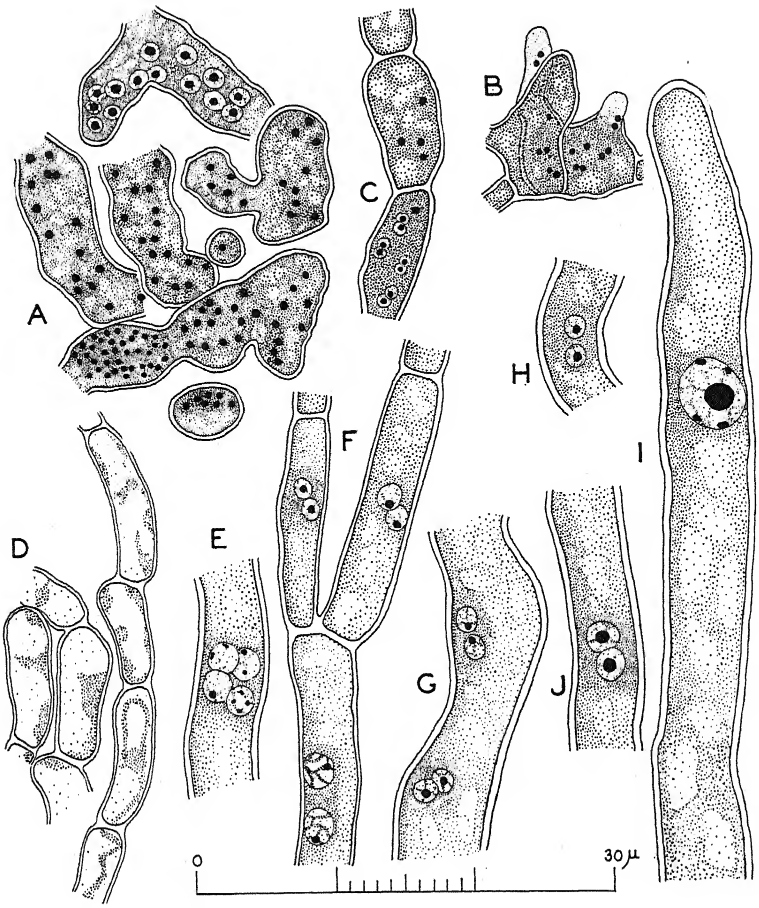

Fig. 4. A, Section through part of an ascogonial coil of an unspermatized receptive body. The contents are densely stained and the nuclei show variation in size and number in the various cells; B, A cell of the ascogonial coil three days after spermatization with compatible microconidia, showing the beginning of the development of ascogenous hyphae and the pairing of the nuclei; C, A similar view four days after spermatization, with further evidence of paired nuclei; D, A comparable section on the sixth day, the ascogonial hyphae are closely septate and there is a marked loss of contents and reduction in the width of the cells; E, F, and G, On the 24th day with the apothecium nearing maturity. Various stages of conjugate division in the ascogenous hyphae. Note the branching in F; H, I, and J, Parts of young asci on the 26th day; H, the nuclei just prior to fusion; I, The fusion nucleus; J, the daughter nuclei from the first division of a fusion nucleus.

or they are held in the depression, submerged by the growth of the surrounding apical hyphae, and met by the elongating trichogynes. The migration of the nucleus from the microconidia and its subsequent passage to the ascogonial coil was not observed.

Receptive bodies spermatized with incompatible microconidia, elongate but slightly, and the structure of the coiled ascogonial hyphae does not exhibit any change even after 15 days. Where compatible microconidia have been used, however, decided modification of the coil is evident at the end of 3 days. The condition of sparse septation is changed to one of frequent septation and each of the resulting cells becomes markedly swollen. During the 4th day the nuclei become definitely paired and the coil gives rise to short protuberances (FIGURE 4 B). The 5th day marks the commencement of the degeneration of the terminal hyphae of the coil, and at the same time the protuberances on the coil can be identified as the branches which are destined to be the ascogenous hyphae. From then onward, rapid development of the ascogenous hyphae occurs, conjugate division of their paired nuclei is evident (FIGURE 4 E, F AND G), and the coiled ascogonial hyphae, on the 7th day, lose their identity through loss of their contents (FIGURE 4 D). Following this, rapid elongation of the apothecial fundament occurs, the apical hyphae gradually take the form of paraphyses, beneath which the ascogenous hyphae are developing. By the 26th day, the apex has begun to expand, the paraphyses are organized into a hymenium and the apices of the much branched ascogenous hyphae are in the subhymenial area. Between the 26th and the 30th day the young asci can be identified as they push their way through the paraphyses. At this stage were observed in the developing ascus the two nuclei prior to fusion (FIGURE 4 H), the fusion nucleus (FIGURE 4 I), and the daughter nuclei resulting from the first division (FIGURE 4 J). The stages between the first division and the early stages in the formation of the ascospores were not evident in the present preparations, but spores that have just been delimited are uninucleate, a condition which persists until just before germination, when this nucleus divides.

No definite statement can be made as to chromosome number. All of the material was killed during the day and the scarcity of

meiotic or mitotic figures in all of the preparations, indicates that the nuclear divisions probably take place at night. These investigations are being continued in the hope that the points now in doubt will be cleared up and that the gaps in the cytological story of this sexual mechanism will be filled in.

THE PROBABLE OPERATION OF THIS SEXUAL MECHANISM IN NATURE

The apothecia, receptive bodies, and microconidia of *S. Gladioli* are developed readily in artificial cultures, but none of these structures has been observed in nature. As stated previously, there is good evidence to believe that the microconidia are formed in the soil in the vicinity of diseased plants, and the presence of the stroma in badly diseased corms indicates that the receptive bodies and apothecia are probably developed from these organs. Favorable conditions of moisture and temperature and the close proximity of compatible thalli would of course be prerequisite factors for the development of apothecia.

The phenomena of self-sterility, and fertility only between compatible forms, leads us to a consideration of possible spermatizing agencies in nature. The mucilaginous material in which the microconidia are embedded would protect them from dessication during periods of drought. This substance is soluble in water, and on account of the minuteness of the spores, they may well be transported through the soil in moving water. Their sticky nature may also allow the sporodochia to adhere to the bodies of soil-inhabiting animals or insects, and so be carried through the soil, possibly to receptive bodies of a compatible thallus.

This fungus is apparently well adapted to prolonged existence in the vegetative stage. The sclerotia will withstand freezing and drought. This, with a possible saprophytic existence in periods favorable for development, enables the fungus to remain alive in soil for many years, even when no susceptible plants are present in the interval. It remains alive in corm lesions during storage, so that the sale and transportation of diseased stock assures dissemination within and between countries and continents.

The fact was previously noted, that in this fungus the resistant structures and the specialized tissue for production of sexually

formed fruiting bodies are separate entities. This is in contrast to the species of *Sclerotinia* having true sclerotia, in which the latter function in both ways. The fact that in other species of *Sclerotinia* the development of receptive structures has never been noted is significant. Apparently in these forms spermatization and fertilization occur before any external development of the sclerotium is evident, and apothecial fundaments then develop when environmental conditions are favorable. The different sequence of events in the case of *S. Gladioli* may be accounted for by the facts that the whole process takes place below ground and that the thalli are self-sterile. With the microconidia borne in the soil, the development of prominent receptive bodies from the diseased corms would greatly enhance the chances of cross-spermatization, especially if soil animals or insects are the principal agency for this purpose. It is possible also that the musty odor emitted by this fungus may have a special attraction for such an agency. This mechanism differs materially from several other species of *Sclerotinia* which must exhibit self-fertility, for cases are on record (**9**, **11**) where apothecia have been obtained from sclerotia developed in monoascosporic cultures. In these cases, the microconidia are borne in contact with the sclerotia, no spermatizing agency is required, and the presence of trichogynous hyphae on the surface of the sclerotia, without any receptive body, would provide an adequate mechanism for fertilization.

The occurrence of this fungus on its various suscepts in their natural habitats has not been recorded. The genera included, comprise plants of both tropical and temperate origin, so that one cannot draw any conclusions as to the climatic zone or suscept in which the fungus may have had its origin. The *Sclerotinia* spp. so far described have been mainly from the temperate zones, but as far as this fungus is concerned, on the gladiolus it seems likely that, in temperate regions at least, the conditions under which it is grown are not favorable for apothecial development. In these regions the corms are harvested in the fall, kept in storage during the winter months and replanted in the early summer, and even if compatible thalli should be present in close proximity and a suitable spermatizing agency present, the temperature conditions while the plants are in the ground would not favor the development

of the sexual stage. In somewhat warmer regions, where it is possible to leave the corms in the field during the winter, the chances of getting apothecia would be greater. The crocus lives the year round in the soil in temperate regions, and it is possible that the sexual stage may develop on this plant, and perhaps the fungus was introduced with it.

It is as yet unprofitable to speculate on the distribution of the two interactive groups of the fungus in nature. In the ten isolates used in this work, the two groups are not segregated on geographic distribution or according to suscepts. In one group there are crocus, freesia, and gladiolus isolates from Holland, Long Island, N. Y., and Indiana respectively. In the other group are two types of gladiolus, and freesia isolates from Holland, Southern Europe, and New York. In any case, no significance could be attached to the source of the diseased material, because of the world-wide movement of corms of all of the suscepts.

DISCUSSION

In the large assemblage comprising the group of sclerotium-producing fungi of ascomycetous affinities, the proportion of the species in which the perfect stage has been found is surprisingly small. This is true, notwithstanding the fact that microconidia, of the type here described, have been observed in many of them, but only a few forms commonly referred to the form-genera *Botrytis* and *Sclerotium* have been connected with species of *Sclerotinia*. From the results obtained in this investigation, it seems probable that many species of these form-genera may in reality possess perfect stages which are as yet unknown because these fungi are self-sterile. It is reasonable to suppose that morphologically identical microconidia occurring in other species may function sexually as do the microconidia of *S. Gladioli*. Yet on the other hand Godfrey (**11**) and Dickson (**9**) report that it is possible to obtain apothecia from monoascosporic cultures of *Sclerotinia Ricini* and *S. sclerotiorum* respectively. In these species, a homothallic condition must exist, but differing from *S. Gladioli* in that they must be self-fertile and the spermatia must be borne in contact with the receptive hyphae, for no spermatizing agency could be operative in undisturbed cultures.

As one attempts to apply experimentally the sexual principles found in *S. Gladioli* to other species, differences in the sexual apparatus will almost certainly be found. For example, clearly differentiated receptive bodies may be exceptional; the trichogynes may be borne singly or in small groups on the surface of sclerotia or stromata, unassociated with any distinctive structure. It is hoped that this investigation will stimulate work along this line and that the apothecial stage of many imperfect forms may be discovered. Such work would undoubtedly assist in clarifying the taxonomic and phylogenetic relationships in this group.

The question of hybridization between distinct species or between physiological forms also enters into this discussion. The sexual function of the pycniospores in the rusts has led to the demonstration of the creation of new pathogenetic races by appropriate combinations of pycnia and sporidial thalli. There is an indication of the presence of pathogenetic races in certain species of *Sclerotinia* and *Botrytis* and it is highly probable that these have been brought about by cross-fertilization.

No reference has been made throughout this paper to the question of heterothallism as applied to the results obtained with *S. Gladioli.* It is felt that the phenomenon to which this term was originally applied is different from the one in *S. Gladioli,* and that the application of the same term here would be incorrect. It was Blakeslee (3) in 1904 who first used this term and defined it when, in discussing the results obtained with *Rhizopus,* he stated, "The condition is similar to that in dioecious plants and animals. . . . Inasmuch, however, as conjugation is possible only through the interaction of two differing thalli, we can express this fact by calling all species the sexual relations of which correspond to the *Rhizopus* type *heterothallic.* In marked contrast to the conditions just described, *Sporodinia* and the other members of the group of which it is the type, invariably reproduce sexually under suitable conditions when grown from a single spore. The zygospores thus originate from a single mycelium, and are comparable to hermaphrodites among the higher plants. Such forms may, therefore, be called *homothallic.*" It seems quite clear from this and later papers that he applied the term heterothallism to a condition in which segregation of sex occurs in such a manner that some

thalli are wholly male, while others are wholly female. It seems reasonable to assume that he would also include in his concept of homothallism, monoecious species in which the thallus is self-sterile.

It is true that the condition existing in *S. Gladioli* is heterothallism in the literal sense of the word. Two groups of thalli exist which differ in a physiological factor which determines the sexual compatibility of the thalli, and it has been shown that segregation of this factor probably takes place in a 1 : 1 ratio. There is no sex segregation, however, for both the microconidia and the receptive bodies consistently develop on each thallus, irrespective of whether these have arisen from single ascospores or from single hyphal tips. The term heterothallism in Blakelee's sense cannot, therefore, be applied to the phenomenon found in *S. Gladioli.*

Miss Cayley (6) points out that sex heterothallism is only one of several forms of heterothallism, and that it is perhaps due to the confusion of these various types that the theory of multiple sexes has gained such a hold. The conception of sterility as the controlling factor in cases similar to the one under discussion was suggested by Brunswik (5), who postulates the existence of two sexes only, the interactions between them being controlled by one or more factors other than those of sex, *i.e.* negative sterility factors. In connection with a discussion of the observations on *Humaria granulata,* Miss Cayley (l. c.) states, "The mycelia must be self-sterile, and although haploid, must be potentially bisexual. They fall into two definite groups in the ratio of 1 : 1, and the members of each group are sterile *inter se*. . . . This is not sex heterothallism, but a form of physiological heterothallism based on one self-sterility factor in a haplo-synoecious fungus." This expresses exactly the interpretation here placed on the situation in *S. Gladioli.*

SUMMARY

Efforts to obtain a sexually formed fruiting body from the minute sclerotia formed by the fungus previously known as *Sclerotium Gladioli* Massey were unsuccessful. Similar trials with the stromatic tissue led to the discovery of structures which proved to be receptive in character, for when these were spermatized with

microconidia from certain other thalli, they developed apothecia of the *Sclerotinia* type.

Monomycelial cultures develop both receptive bodies and microconidia, and ten isolates thus derived from various localities and suscepts were crossed in all possible ways and the development or lack of development of apothecia in the various crosses was tabulated. This experiment demonstrated the fact that the isolates used could be divided into two groups on the basis of compatibility, and these exhibit intra-group sterility, inter-group fertility, and individual self-sterility.

Single ascospore cultures were made, the receptive bodies from these were back-crossed separately with microconidia from each of the parent isolates, and this showed clearly a segregation of the compatibility factor, apparently in the ratio of 1 : 1.

Other experiments demonstrated that receptive bodies could be fertilized by microconidia developing on ascospores which had been discharged on the receptive bodies; that a filtered suspension of microconidia in which the latter were removed, did not effect fertilization, and that apothecia would not develop as the result of hyphal fusions, even between compatible isolates.

Optimum cultural conditions for the development of microconidia and receptive bodies are outlined, and also the technique used for the process of spermatization and the development of the apothecia. Experiments were performed which demonstrated that the receptive bodies are the only structures capable of fertilization by the microconidia.

Some preliminary cytological notes are given, including details of the changes which take place in the coiled ascogonial hyphae of the receptive bodies, when the later are spermatized with compatible microconidia.

Some speculation is made on the operation of this sexual mechanism in nature, for the microconidia, receptive bodies, and apothecia have been seen only in artificial cultures.

The phenomenon exhibited by this sexual mechanism is not regarded as one of heterothallism, as Blakeslee defined this term, for there is no segregation of the sexes in separate thalli, but rather a homothallic condition in which each thallus is self-sterile and fertility exhibited only between certain compatible thalli.

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EXPLANATION OF PLATES

PLATE 5

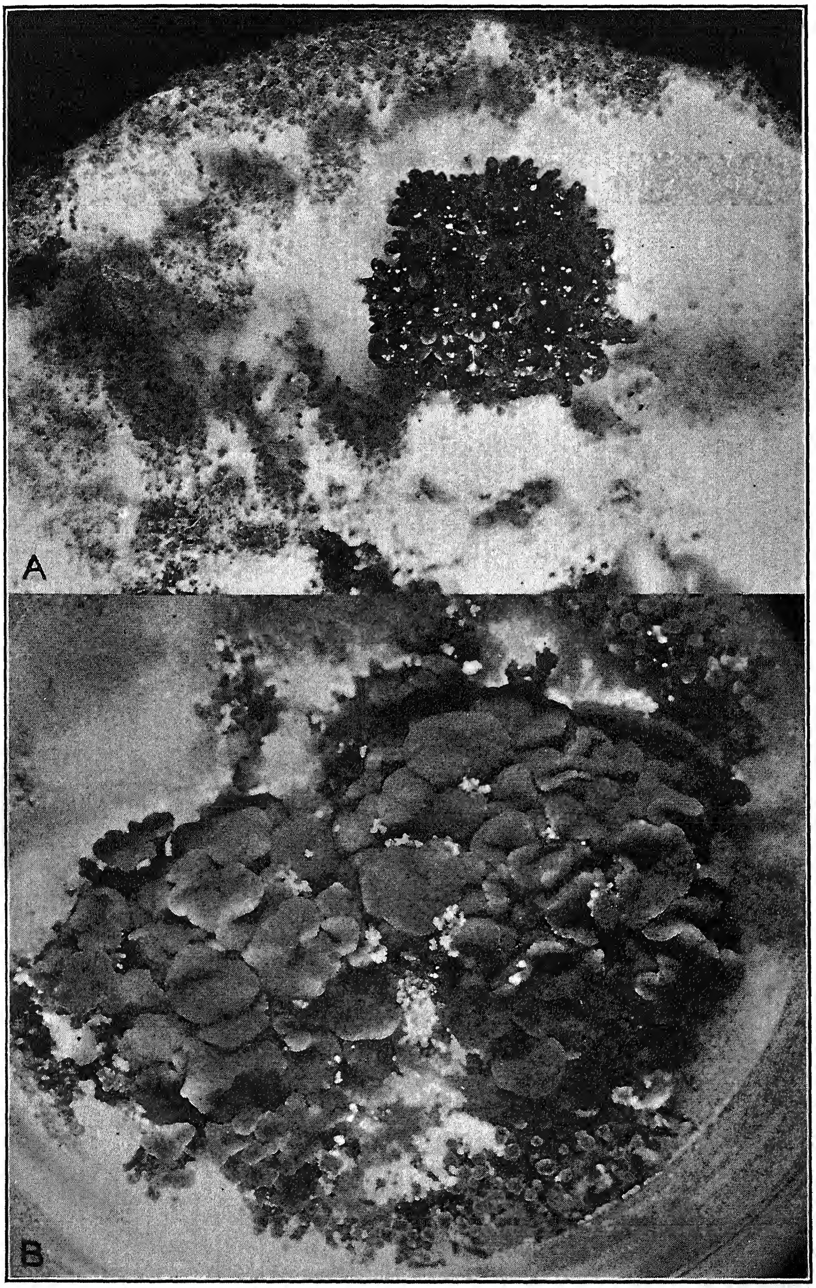
A, The crocus isolate growing on a sterilized gladiolus corm. Receptive bodies have formed and two groups (one only showing) have been spermatized with compatible microconidia. Note the apothecial fundaments developing in the spermatized area; the remaining receptive bodies continuing unchanged, × 4; *B,* a larger area of the culture shown in *A,* three weeks later. The apothecia from the spermatized groups are now mature. The originally unspermatized receptive bodies, now spermatized by microconidia formed by the ascospores shot from the previously matured apothecia, are forming apothecia, × 3.

PLATE 6

A, A test culture in a Petri dish, the right half spermatized with compatible microconidia, the left half treated with soil extract alone, natural size; *B,* A six weeks old *Lycium* stem-potato-dextrose agar culture. Note the sclerotia in the foreground, and three receptive bodies in the background with microconidial sporodochia formed on them, × 8.

PLATE 7

A, A longitudinal section of a receptive body, showing the stromatic base and part of its rind, the pilose exterior, the sterile apical hyphae, and the central system of coiled ascogonial hyphae, × 70; *B,* The apex enlarged to show the overarching of the sterile hyphae and the depression formed at the center, × 210; *C,* Part of the coiled ascogonial system showing the intricacy of the coiling, × 2000; *D,* Section through portion of a coil showing their deep staining character and the nuclei, × 2250; *E,* Section through the stroma, showing the loose structure of the rind, and the coiled ascogonial hyphae beneath the surface prior to the development of a receptive body, × 1000.



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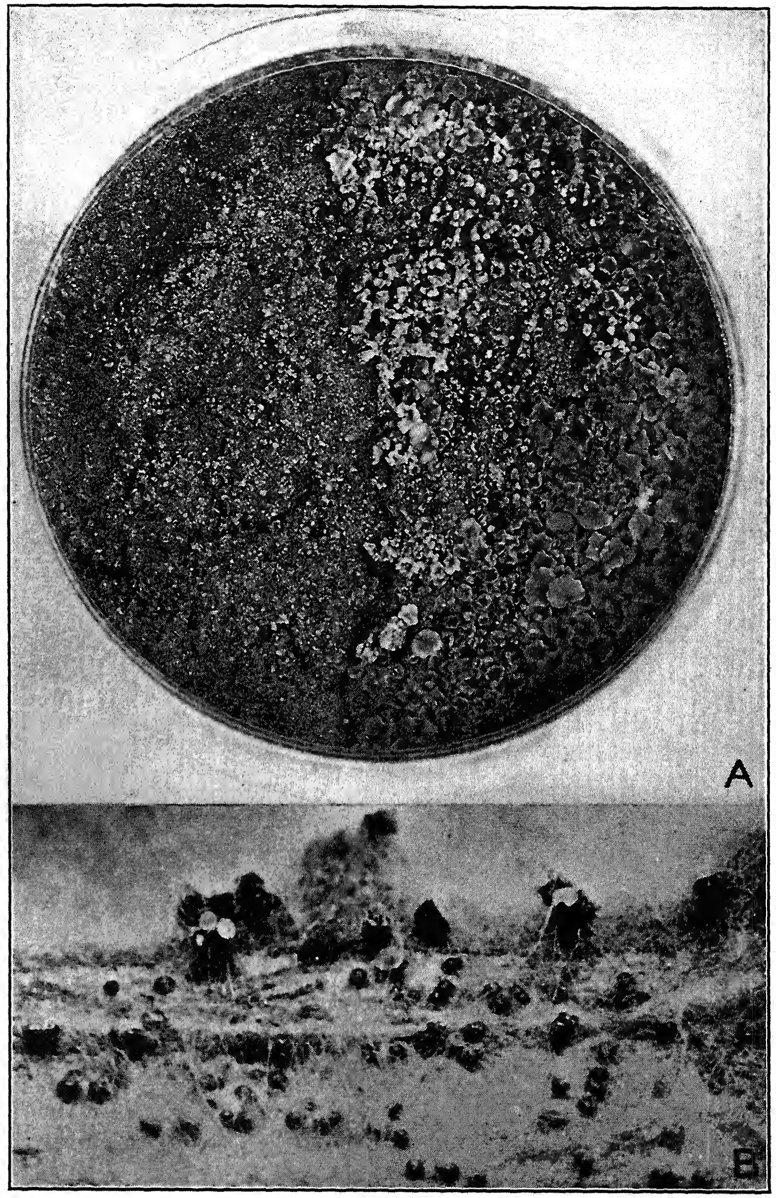

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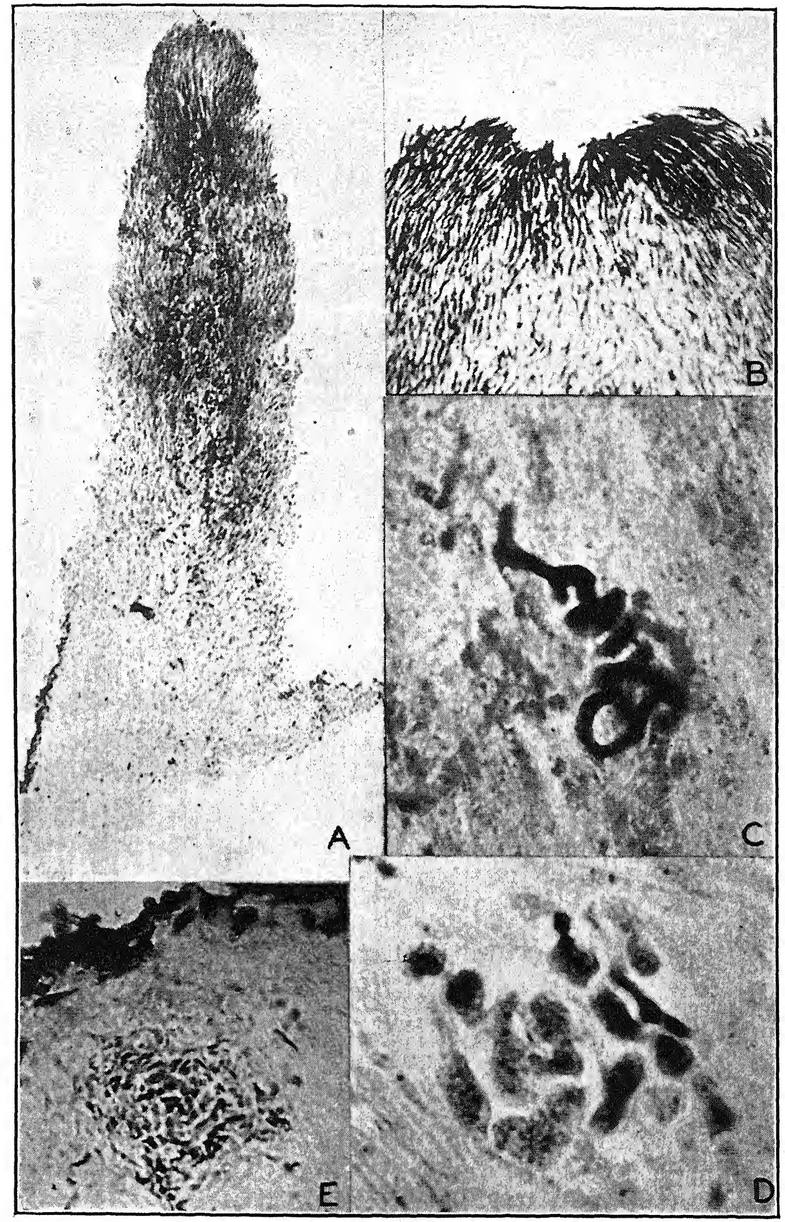


Sclerotinia Gladioli

DASYSCYPHAE ON CONIFERS IN NORTH AMERICA. I. THE LARGE-SPORED, WHITE-EXCIPLED SPECIES

Glenn Gardner Hahn and Theodore T. Ayers

(with plates 8–13)

INTRODUCTION

With the discovery of the European larch canker on introduced *Larix europaea* D.C. in Massachusetts in 1927, and the subsequent diagnosis of the introduced causal organism as *Dasyscypha calycinia* (Schum.) Fuckel by Spaulding and Siggers (following general British usage) (13), extensive scouting for the disease was performed to determine whether the parasite was widely distributed. A pathological investigation of the disease was also undertaken particularly with reference to the host relationships of the causal fungus.

On account of the considerable nomenclatorial confusion in the literature brought about by the variety of names which had been applied in Europe to the larch canker parasite since the middle of the 19th Century, there immediately arose the pressing mycological problem, in this case of particular economic concern, having to do with the exact morphological determination of the larch canker organism. This confusion was increased by the fact that in North America the European parasite had been identified as being present previous to the actual discovery of typical cankers on imported larch in this country. It therefore became necessary to recognize this organism in order that its presence and spread in this country on larch and other conifers might be determined with a high degree of certainty. The fact that in Europe the parasite had been reported on pine and Douglas fir, caused considerable concern when related *Dasyscypha* forms were discovered (7) on cankered Douglas fir [*Pseudotsuga taxifolia* (LaM.) Brit., blue form] in New England. These diseased Douglas firs in certain instances were growing in close association with the imported larch. Ac-

cordingly it became highly important to ascertain whether the imported parasite constituted a potential menace to Douglas fir in this country.

The extensive scouting for the disease among conifers in New England and the Middle West produced numerous collections of *Dasyscypha*. These were augmented by other collections taken in the South and on the Pacific Coast. The authors were thus able to study morphologically and culturally a comparatively large number of forms collected throughout the United States and in Canada.

In addition to collections of the European larch canker organism, fresh material of the following coniferous parasites were made available for study: *Dasyscypha fuscosanguinea* Rehm, *D. resinaria* (Cooke & Phil.) Rehm, *D. Ellisiana* (Rehm) Sacc. (the newly discovered parasite of Douglas fir), and *Dasyscypha* forms nearly related to *D. calyciformis* (Willd.) Rehm.

It is the purpose of the writers to publish their observations on North American *Dasyscyphae* on conifers in a series of papers of which the present one is the first. In this publication the large-spored, white-excipled forms are dealt with. The native species are differentiated from the economically important introduced parasite, *Dasyscypha Willkommii* (Hartig) Rehm, (the Continental name for the European larch canker fungus, accepted by the authors), and the European saprophyte, *D. calycina*. Pathological data used to corroborate physiological differences among the species studied, are only briefly alluded to, and will be reported in detail elsewhere.

THE GENUS DASYSCYPHA

The taxonomy of *Dasyscypha* (1869) has been the subject of controversy due to the acceptance of Karsten's genus, *Lachnum* (1871). Adhering to priority of position as published, *Dasyscypha* Fuckel (2)with its type, *D. bicolor* (Bull.) Fuckel, which was the first species described under the new genus, should replace *Lachnum* Karst. (8), containing species with paraphyses "apice acutae vel acutatae aut saltem attenuatae, discretae." *D. bicolor* is recognized as having broad, acerose paraphyses. Unfortunately when Fuckel raised the Friesian (Syst. Myc. 2: 89,

1822) tribal name, "Dasyscyphae" (δασύς, hairy; σκύφος, a cup), of the large genus *Peziza,* to generic rank, he briefly described his new genus without mentioning the morphological characters of the paraphyses. Of the seven species listed by Fuckel under his new genus, only one, *D. calycina,* had filamentous paraphyses. For reasons outlined above, Boudier (Bull. Soc. Myc. Fr. **1**: 117. 1885) reserved the Fuckelian name, *Dasyscypha,* for the hairy-stalked forms with broad, lance-shaped paraphyses, wherein he placed *D. bicolor* and *D. cerina* (Pers.) Fuckel, whereas *D. calycina* was referred to his new genus, *Trichoscypha.*

Fuckel's genus *Dasyscypha* was recently discussed by Nannfeldt (**9**, pp. 298–299) who, although he admitted the priority claim of *Dasyscypha,* believed that the retention of Fuckel's genus for species with filamentous paraphyses would only lead to confusion. Accordingly he would retain the distinct generic name *Lachnum* of Retzius (Fl. Scand. Prodr. 329. 1779), and of Karsten (**8**), for forms with lanciform paraphyses, and do away entirely with the name *Dasyscypha.* Since Nannfeldt found that *Trichoscypha* of Boudier was a homonym of the older *Trichoscypha* Hook., he substituted his new name, *Trichoscyphella* [*T. Willkommii* (Hartig) Nannf.].

The confusion which Nannfeldt would avoid appears to be added to by this procedure in discarding *Dasyscypha.* It is not the purpose of the authors to argue the point. They are of the opinion that because of the long-time usage of *Dasyscypha* and the universal recognition of the genus, it should be retained. Following Rehm, and Clements and Shear (The Genera of Fungi. 1931), they have allocated to *Dasyscypha* the hairy-cup coniferous Discomycetes, all of which were found to have filamentous paraphyses, with extremities unswollen, obtuse or subacute, or slightly swollen. It is here suggested, however, that inasmuch as the pre-Friesian genus, *Lachnum* Retz., accepted by Rehm and others, has been employed to indicate forms having lance-shaped, "vulgo grandiusculae" paraphyses (**8**), it would seem proper to accept Fuckel's second-named species, *D. calycina,* as the type of *Dasyscypha.*

There has been considerable discussion over the identity of Fuckel's *Dasyscypha calycina* (Schum.) and its relationship to

Peziza calycina Schum. and *P. calycina* Fries. For reasons given in the following section the Fuckelian species is upheld.

HISTORY AND DESCRIPTION OF DASYSCYPHA CALYCINA

When Fries (1822) introduced the tribe " Dasyscyphae " he included the species *Peziza calycina* despite the fact that Schumacher (Enum. Pl. Saell. **2**: 424. 1803) had previously occupied that name with a Discomycete collected " in strobylo *Pini abietis*." Fries described three forms of his species:

α. *Pini sylvestris* (Syst. Myc. **2**: 91, 1822) = *P. calyciformis* Willd.; *P. calycina* Schum., " in ramis *Pini silvestris*."
β. *Abietis*
γ. *Laricis* (Elench. Fung. **2**: 8. 1828) " in ramis, *Pini laricis*, Chaillet."

The description of the first-named form, *Pini sylvestris*, which was given in greater detail than the others, is nevertheless inadequate in revealing to us just what fungus on pine Fries actually intended. Undoubtedly he was dealing with a heterogeneous group of fungi on pine, fir and larch. His first form, *Pini sylvestris*, may or may not have been *D. calyciformis* (Willd.) Rehm with short, elliptical spores. Because of the common occurrence of this saprophyte in Europe on pine, spruce and fir, it is quite possible that Fries was concerned with this fungus but of this we are not sure. No spore measurements were given by him or by Schumacher for his fungus, the type of which is now known to be lost.

In 1869 Fuckel (2) definitely occupied the name *Dasyscypha calycina* with a large-spored fungus " an dürren berindeten Aesten von *Larix europaea* " for which he was the first to give spore measurements. It is not at all probable that his fungus was the same as Schumacher's on fir cones; for the large-spored *Dasyscyphae*, as this paper will show, are extremely limited in their host range.

It is a convention among mycologists to accept Fries' names for Ascomycetes where these are sufficiently described. Where a species is insufficiently delineated it has been the custom to take the name given by the first investigator after him who gave a description on which a species could be based. Fuckel was the first after

Fries to adequately occupy the name, *Dasyscypha calycina* with a fungus, which from his description, we can consider saprophytic on larch.

Opportunity was afforded to examine a herbarium specimen, Fungi Rhen. *1206, D. calycina* Fuckel, from the Herbier Barbey-Boissier at Geneva (PLATE 8, FIG. 1). This collection, which was issued by Fuckel in 1864 previous to the publication of his original description (2), was acquired in 1894 by Barbey-Boissier after Fuckel's death (1894), and distributed by them. The Geneva specimen contained abundant ascocarps and these, although considerably weathered by long-keeping, showed spores agreeing in size with those described by Fuckel.

An amended description of *D. calycina* which follows, attempts to clearly define that species. In doing so, we hope to terminate the controversy over the relationship between this saprophyte, which we found to be non-pathogenic, and the parasite of Berkeley, Willkomm and Hartig. Our evidence supports in part the Continental school who have maintained, contrary to the British point of view,[1] *D. Willkommii* to be a distinct species. It does not support, however, the Continental usage of Fries' name "*calycina*" to designate a short-spored *Dasyscypha* on pine and other coniferous hosts, for the reason that the name "*calycina*," as we have endeavored to demonstrate, is definitely occupied by a much larger-spored fungus which Fuckel intended, on larch.

DASYSCYPHA CALYCINA Fuckel Symb. Myc. 305. 1869. Descr. emend. nec *Peziza calycina* Schum. Enum. Pl. Saell. **2**: 424. 1803.

Ascocarps abundant, solitary or grouped, with short stalks, erumpent, at first globular, closed, opening in a rounded form, and

[1] What may be called the British contention is that *Dasyscypha calycina* (Schum.) Fuckel is the proper name by which the European larch canker parasite should be recognized (6). However, this view has not been held by all British workers, *e.g.*, H. M. Ward (Timber and some of its diseases 227–243. 1889) was of the following opinion: "At the margins of the flattened patch, just where the dead cortex joins the normal living parts, there may frequently be seen a number of small cup-like fungus fructifications, each of which is white or grey on the outside, and lined with orange-yellow. These are the fruit bodies of a discomycetous fungus called *Peziza willkommii* (Hartig), and which has at various times, and by various observers, received at least four other names, which we may neglect."

expanding with humid conditions to a flattened, saucer-like structure with a comparatively thin rim (PLATE 8, FIG. 2); externally whitish with cylindrical, thin-walled hairs, minutely roughened, with gently rounded, slightly swollen extremities, septated, cells short, 3–4 μ broad, hairs persistent (PLATE 9, FIG. 8), disc ochraceous-salmon[2] to salmon-orange 0.5–3 mm. diam; asci clavate, frequently swollen toward the apex with obtusely-rounded apices, range (198) 82.8–165.6 $\times$ 6.8–12.8 μ, commonly, 100–130 $\times$ 8–10 μ (PLATE 9, FIG. 6). Ascospores eight, uniseriate, continuous at first, commonly uniseptate upon germination, smooth, hyaline, elongate-elliptic or elliptic-oblong, very rarely fusiform or pointed at one end, range (340) 12.6–21.4 $\times$ 4.2–8.4 μ, commonly, 14–19 $\times$ 5–7 μ (PLATE 9, FIG. 7); paraphyses outranking asci, variable, slender, filamentous, unswollen at the extremities, commonly intermixed with broader filaments, irregularly submoniliform, with swellings near their extremities, obtusely rounded or attenuate with subacute apices, occasionally spathulate, septate, range (155) 90–180 $\times$ 1–4 μ (PLATE 9, FIG. 6).

Imperfect stage consisting of erumpent, fleshy, waxy stromata containing simple or labyrinthiform loculi in which microconidia (spermatia) are abstricted from the tips of short, subulate sporophores, simple or verticillately-branched (PLATE 9, FIG. 9); microconidia continuous, hyaline, elliptic-oblong or allantoid (100) 2–5 $\times$ 1–2 μ (PLATE 9, FIG. 10). Germination not observed.

Type—Fungi Rhen. *1206*. (Barbey-Boissier, Herbier Boissier *1316*, Geneva.)

The fungus has been observed as a saprophyte in Massachusetts where the European larch canker parasite was introduced. The following collections from the Matthews Estate, Hamilton, Mass., made in 1929–1931 have been studied.[3]

On *Larix europaea*.—*43536*, coll. G. G. Hahn & J. R. Hansbrough; *43561–62*, coll. H. Metcalf, P. Spaulding, N. O. Howard & Hahn; *43564–65*, coll. Hahn; *53057*, coll. Hahn, T. T. Ayers & C. S. Moses; *53067*, coll. Ayers; *53131*, coll. Hahn; *53701*, coll. Hahn & Ayers.

On *Larix leptolepis* Gordon.—*53087*, coll. Hahn; *53702*, coll. Hahn & Ayers.

On *Pseudotsuga taxifolia*, blue form,—*53056*, coll. Ayers. An

[2] The color nomenclature used is that of R. Ridgway, Color standards and color nomenclature, 1912, Washington, D. C.

[3] Unless otherwise indicated, collection numbers denote specimens for study filed in the Division of Forest Pathology, New Haven, Conn.

intensive search in 1930–1932 showed that *D. calycina* was exceedingly rare on this host. Previously on July 17, 1927, Siggers (13) found a scant collection (*53883*) of the species occurring saprophytically on a twig of blue Douglas fir growing in the Hamilton, Mass. area. A mono-ascus strain of *D. calycina* isolated from the Douglas fir collection, *53056,* and inoculated into a dying blue Douglas fir (August 13, 1931) produced numerous apothecia (1933) about the incision, the paraphyses of which were submoniliform (PLATE 9, FIG. 6), being characteristic of those recognized as typical for the species.

In Scotland the senior author found *D. calycina* occurring occasionally as a saprophyte on the green form of Douglas fir growing in a mixed planting with badly diseased European larch. Despite the crowded condition of the stand on a site favorable to the development of larch canker, it was observed that the Douglas fir was not affected by the larch canker organism (*D. Willkommii*). However, collections of *D. calycina* were made on both *L. europaea* and *Pseudotsuga taxifolia.*

Ascocarps of *D. calycina* on Douglas fir in Scotland collected during the spring and summer (1927 and 1929) on weakened, partly living, or dead Douglas fir branches and twigs, were never so abundant as those found upon larch. The following Scottish collections were made at Glentress, Peeblesshire: *43510–33–34, 43998.*

Scottish examples of the fungus have also been identified in the herbarium of Dr. J. S. Boyce, among them: *1607, Larix eurolepis* Henry, and *1606, L. europaea,* Dunkeld, coll. J. S. Boyce, Aug. 11, 1925; *1603, Pseudotsuga taxifolia,* Bowmont near Kelso, coll. J. S. B., Aug. 19, 1925.

Among fresh specimens of *D. calycina* on *Larix europaea* which have been studied from Great Britain and the Continent are the following: *43500–6–7–20,* Glentress, Peeblesshire, Scotland, coll. Hahn, June, 1929; *53128,* Yorebridge, Scotland, coll. M. J. F. Gregor, Feb. 7, 1931; *53130,* Hann.-Münden, coll. E. Plassmann, Sept. 1930; *53158* (on upper edge of canker in which *D. Willkommii 53159* was fruiting), West Linton, Scotland, coll. Gregor, May 3, 1931; 53811, Forêt du Rac Estable, Axat, Aude, France, coll. G. Fenwick-Owen & G. D. Darker (*3956*) Mar. 2, 1932;

53829, Zürichberg, Zurich, coll. G. D. D. (*4093*) June 15, 1932; *53836,* Nový Smokovee, Czechoslovakia, coll. G. D. D. (*4187*) July 18, 1932; *53837,* Vimperk (Winterberg), Czechoslovakia, coll. G. D. D. (*4136*) July 2, 1932.

Exsiccata examined:

Fungi Rhen. *1206, Dasyscypha calycina* (Schum.) Fuckel. (Herb. Kew) is without apothecia.

Fungi Rhen. *1206, D. calycina* (Schum.) Fuckel. 1864 (Herb. Bot. Mus. Berlin—"sehr spärliches Material" according to a written communication from Dr. Ulbrich) is neither *D. calycina* nor *D. Willkommii,* but a closely related form with blunt, elliptic spores, 11–17 × 5.5–7 μ and fine filamentous paraphyses mostly unswollen or very slightly so at the tip. The ascocarps are quite minute.

Fungi Rhen. *1206, Dasyscypha* (*Peziza*) *calycina* (Schum.) Fuckel. 1864 (Farlow Herb.) is possibly identical with the Berlin specimen. The material is exceedingly meagre and weathered, spores 12–16 × 4–5.5 μ and filamentous paraphyses mostly unswollen at the tip. Fungi Rhen. *1206, 1864* (*1316,* Barb. Bois., 1894), from the same herbarium, is a small-spored form, 5–8.5 μ long belonging to the *D. calyciformis* (Willd.) Rehm group.

Fungi Rhen. *1206, D. calycina* (Schum.) Fuckel. 1864 (*1316,* Herb. Bois., 1894) in Myc. Herb., U. S. D. A., Washington, D. C., is also exceedingly meagre. Slides of one ascocarp did not reveal any morphological detail. A slide of a second ascocarp prepared in 1928 by W. W. Diehl showed only a very few elongate-elliptic spores, several of which were fusiform, 14–21 × 5–7 μ, associated with filamentous, unswollen paraphyses. Although apparently closely related, it is probably not *D. calycina* because of the absence of the submoniliform paraphyses.

We were very fortunate in obtaining the Geneva material for the foregoing studies of the type *D. calycina.* In our search for type material of *D. Willkommii* we were not so fortunate. Our observations on this species have been made largely from fresh European and American material.

HISTORY AND DESCRIPTION OF DASYSCYPHA WILLKOMMII

The European larch was introduced into the lowlands of Germany from its native habitat about the beginning of the 18th Century. There it appeared to thrive successfully in artificial plantations. About the middle of the following century there appeared a serious canker disease among the planted larch, which in certain areas caused considerable damage by deforming and killing young stock.

For a hundred years there occurred continual strife in the literature as to the cause of the larch canker. At first the articles on the disease were largely pathological in nature. It was Hiley (6) who pointed out that in 1859 Berkeley (Gard. Chron. 1015–1016) was the first to ascribe the cause of the canker to a fungus. In a specimen, "forwarded by Sir Walter C. Trevelyan," Berkeley found that "mycelium has penetrated through the bark and produced its proper fungus, under the form of *Peziza calycina*." He went on to relate that, "In a small plantation, most of the trees of which are young, nearly all are more or less attacked on stem or branch with the *Peziza*." Seven years later Willkomm (**14**) published his treatise setting forth in great detail the symptoms and etiology of the disease and ascribed the cause to a Discomycete which he profusely illustrated but incorrectly called *Corticium amorphum* (Pers.) Fries. He came by this error as the result of blindly accepting a determination of the European larch canker organism as *C. amorphum*. This determination had been made by Rabenhorst apparently in haste and without critical observation. It is a well-known fact that this Basidiomycete superficially resembles certain *Dasyscypha* forms to such an extent that they can be readily confused, *e.g.*, a specimen of *Peziza calycina* Fries *β abietis*, Fungi Rhen. *2192*, 1868, in the Farlow Herbarium, Harvard University, is not a *Dasyscypha* but *Aleurodiscus amorphus* (Pers.) Rabenh. (*Corticium amorphum*).

Hoffman (Forst-u. Jagd-Zeit., May, 1868) corrected Willkomm's error and adopted the same name for the fungus as Berkeley. Robert Hartig (4) likewise recognized Willkomm's mistake, but in correcting the error he made a new name, *Peziza Willkommii* Hartig in honor of the forest pathologist, Willkomm, for he believed the larch canker parasite to be a new species. In

commenting upon the correction made by Hoffman, which Hartig regarded as an error, he stated that among the varieties of *P. calycina* based upon host differences as established by Fries, he (Hartig) recognized well-defined differential characters in the size and shape of the ascospores and the size of the asci and paraphyses. In the case of *P. calycina* Fries, Hartig understood a short-spored form on pine and fir, that was probably identical with *Dasyscypha* (*Peziza*) *calyciformis,* the species recognized not only by Rehm but also by mycologists of the present day. Hartig added very little to the morphological detail of the larch parasite. As in the case of Willkomm, he was largely concerned with the disease caused by the organism and in his brief description Hartig referred to the morphological data given in Willkomm's paper. He (5) illustrated a fungus with large, elliptic-fusiform spores and filamentous paraphyses, unswollen at their apices. Apparently neither Willkomm nor Hartig distinguished between the parasitic and saprophytic forms on larch. There is no doubt, however, that they were largely concerned with the fungus fruiting "besonders an Krebstellen" (4, 5), which was causing so much discussion at that time.

In 1889 Saccardo (Syll. Fung. 8: 437) placed Hartig's new fungus as a synonym of *Dasyscypha calycina* Fuckel. He described a form with large spores and filiform paraphyses "sursum incrassatulis," occurring on larch and Scotch pine in Britain, Germany, France and Italy. Saccardo did not mention the presence of elongate-fusiform pointed ascospores.

Rehm in 1871 issued a specimen (Rehm, Ascom. *62*), *Dasyscypha calycina* (Schum.) Fuckel, collected by himself. Linhart referred to this collection when in 1882 he issued Fungi Hung. *62,* to which he applied the combination, *Dasyscypha Willkommii* (Hartig) Rehm.

Carruthers (1) in 1891 used this same binomial independently for the organism causing canker of the larch. In an exceedingly comprehensive paper dealing largely with the disease, Carruthers stated: "The structural characteristics are no doubt sufficient to separate this fungus (the hitherto unnamed plant figured by Willkomm which Hartig designated as *Peziza Willkommii*) as a distinct species, apart from the consideration that it attacks only the

living larch, while the *Dasyscypha calycina* is found on the dead branches of the Scotch fir . . . the plant must now be called *Dasyscypha Willkommii*." Carruthers, judging from statements made concerning his pathological observations, was firmly of the opinion that the larch canker fungus was essentially a parasite of very young active bark, which it was able to penetrate causing fresh cankers by new and independent attacks. He figured a fungus with large, elliptical spores associated with filamentous paraphyses unswollen at the tip. Without a doubt Carruthers was concerned with the true larch canker parasite despite the fact that he failed to note the pointed spores which are so characteristic of the parasite. He regarded *D. calycina* as quite a distinct species on pine.

In 1896 Rehm (12) published the first complete description of *D. Willkommii* (Hartig) with illustrations of a large-spored form with elongate-elliptical and spindle-shaped, pointed spores, associated with filamentous paraphyses unswollen at the tip as figured by Hartig (**5**). Rehm erred, however, in regarding *D. calycina* Fuckel (Fungi Rhen. *1206*) as a synonym, showing the general tendency of the time not to recognize specifically a closely related but distinct, large-spored saprophytic form.

A preliminary study in the field and laboratory in Scotland in 1929 convinced the senior author that the European larch canker organism could be distinguished morphologically and physiologically from nearly related saprophytic forms. A continuation of this study among diseased imported larches in the United States showed conclusively that the imported parasite was a distinct species.

[4] The variety *Fuckelii* Bresad.,—syn. *Dasyscypha calycina* var. *minor* Rehm (Ascom. Lojk. 8, 1882), of *D. Willkommii* was proposed by Rehm anew in 1896 (12, p. 833) for a specimen in his herbarium. He described this varietal form as follows: "Schläuche cylindrisch, 90–100 μ long, 7–8 μ breit. Sporen elliptisch, stumpf, 10–15 μ long, 5–6 μ breit. An dürren Aesten von Lärchen . . . (Bres.), von *Pinus pumilio* . . . (Britzelmayr), . . . Bresadola bezeichnete seinen Pilz als synonym zu *Dasyscypha calycina* Fuckel, nec Schum. Derselbe ist aber doch nur als kleinsporige Form von *D. Willkommii*. . . ." Previously Bresadola commented upon a small-spored fungus, *Dasyscypha calicina* (Hedw.) Fries, (Roum. Rev. Myc. **13**: pt. 49, 23, 1891), "sur les branches de l'*Abies excelsa*," but he did not mention a variety *Fuckelii*. Until the authors have examined Rehm's specimen in order to determine the character of its paraphyses which he did not de-

The following description of *D. Willkommii* will serve to separate the parasite from the nearly related large-spored saprophyte *D. calycina*.

DASYSCYPHA WILLKOMMII (Hartig) Rehm in Ber. Naturh. Ver. Augsburg **26**: 19. 1881; Rab. Krypt.-Fl. **1**^{3}: 832. 1896.[4] Syn. *Peziza Willkommii* Hartig.

Trichoscyphella Willkommii (Hartig) Nannfeldt.

Apothecia very robust (PLATE 10, FIGS. 2, 3), waxy, fleshy, scattered or grouped, erumpent, at first globular and closed, then opening urn-like and expanding saucer-shape, disc bulging in a convex manner forming a convoluted hymenial surface with an irregular periphery; rim not becoming laterally compressed on drying, comparatively thick and splitting in a cruciform manner under moist conditions; short but distinctly stipitate; externally chalky-white (PLATE 10, FIG. 2), covered densely with excipular hairs, minutely roughened, hyaline, thin-walled, cylindrical with slightly swollen, gently-rounded extremities, septated, forming short cells, 3–4 μ broad, persistent (PLATE 9, FIG. 3); disc apricot-orange or apricot-buff to salmon-buff fading to light buff, 3–6 mm. diameter. Asci clavate, apex obtusely rounded, range (205) 126.0–172.8 $\times$ 9.0–14.0 μ, commonly, 140–162 $\times$ 10–13 μ (PLATE 9, FIG. 1). Ascospores eight, obliquely uniseriate, hyaline, smooth, continuous at first commonly uniseptate upon germination, elongate-elliptical, fusiform, obtuse or acute extremities, or acute at one end, range (200) 15.6–27.8 $\times$ 6.0–9.4 μ, commonly, 17–25 $\times$ 7–9 μ (PLATE 9, FIG. 2). Paraphyses outranking asci, flexuous-filiform, generally not swollen toward the tip, or only very slightly so, with obtuse extremities, septate, range (50) 158.4–216.0 $\times$ 1–3 μ, extremities 1–4 μ broad (PLATE 9, FIG. 1).

The conidial which precedes the apothecial stage consists of waxy, fleshy, erumpent, whitish stromata with irregular labyrinthiform cavities in which the microconidia (spermatia) are abstricted from the tips of slender, subulate, acutely-pointed, simple or verticiallately-branched sporophores (PLATE 9, FIG. 4); microcondidia (PLATE 9, FIG. 5), hyaline, continuous, elliptic-oblong, or allantoid, with obtuse extremities, (100) 2–8 $\times$ 1–2 μ [Hartig (**5**) *pl. 11, fig. 16, 17, 18;* Plassmann (**10**, p. 14) fig. 5; Gaisberg (**3**), *pl. V, fig. 13, 14*]. Germination was tested repeatedly but was unsuccessful.

scribe, and have compared cultures made from fresh collections of this European variety, they cannot state whether it is identical with their new North American species, *D. occidentalis*. Further study may show the European variety to be a distinct species.

Dasyscypha Willkommii is only to be found in immediate association with the lesions (PLATE 10, FIG. 1) which it causes. Later *D. calycina,* among other saprophytes, may come in about the canker after the vigor of the branch part attacked, has greatly declined or the branch is dead, spreading prolifically throughout the dead tissue.

Numerous specimens of the typical European larch canker organism have been collected (1927–1931) by members of the Division of Forest Pathology on *Larix europaea* which host was introduced from Great Britain into Massachusetts upon the Palmer Estate, Ipswich, and the Matthews Estate, Hamilton, in a diseased condition.

The organism was also found fruiting very sparsely upon lightly cankered *Larix leptolepis* which was also imported from Great Britain.

The senior author made numerous collections of typical *D. Willkommii* in Scotland and Norway which agree morphologically with specimens collected in the United States. A specimen of the fungus collected on *L. europaca* at Weissenbach, Austria, by Dr. E. P. Meinecke, July 16, 1914 (Herb. E. P. M.) was found to be identical with the American material.

D. Willkommii is reported to cause larch canker not only upon *L. europaea* and *L. leptolepis,* but also upon *L. occidentalis* Nutt., *L. sibirica* Led., *L. laricina* (Du Roi) Koch, and *L. dahurica* Turcz. in Europe. The authors have been able to infect artificially *L. laricina, L. europaea* and *L. leptolepis* with the fungus in the United States. Spaulding in a verbal communication stated that in May, 1922, he observed both the native American larches, *L. laricina* and *L. occidentalis,* infected with the European larch canker disease in Bagley Wood, England.

Exsiccata examined:

An effort was made to secure some of Hartig's original material of *Peziza Willkommii.* Through the kindness of Dr. Mary J. F. Gregor, Edinburgh, Scotland, information was obtained from Dr. K. von Tubeuf concerning type material of *D. Willkommii* collected by Hartig at the time of his first description. It was, however, not possible to obtain material for study of this period for reasons stated by Tubeuf in correspondence,—" . . . Die 1 Bes-

chreibung Hartigs (Wichtige Krankheiten der Waldbäume) ist in Eberswalde bei Berlin gemacht vor 1879. Diese Sammlung ist grösstenteils oder ganz zur Zeit Brefelds durch Annobien zerstört worden."

Rehm, Ascom. *62b, Dasyscypha Willkommii,* coll. J. Rick, 1898 (at Farlow Herb.) appears to be the larch canker parasite; Rehm, Ascom. *62b, D. Willkommii* Hartig, coll. Magnus, 1892 (at Farlow Herb.) is not *D. Willkommii* but a related large spore form. The paraphyses appeared to be swollen at the tips but these were so degenerate that it was impossible to tell much about them.

Kunze, Fungi Sel. *383, D. Willkommii* (Hartig) Winter,—syn. *Peziza Willkommii* Hart., coll. G. Winter, *1878* (Farlow Herb.) is neither *D. Willkommii* nor *D. calycina,* but a distinct form.

Linhart, Fungi Hung. *62, D. Willkommii* (Hartig), cfr. Rehm, Ascom. *62, Peziza Willkommii* Hartig, 1882 (Herb. E. P. Meinecke) does not appear to be *D. Willkommii* but possibly *D. calycina.* The rather poor, large-spore material showed comparatively broad paraphyses, some of which were swollen toward the tip.

TWO UNDESCRIBED NORTH AMERICAN DASYSCYPHA SPECIES

Both *Dasyscypha calycina* and *D. Willkommii* have been reported by mycologists as occurring in North America before these two European species were actually demonstrated as being present on imported larch. Kauffman (Unreported Michigan Fungi for 1909, Rept. Mich. Acad. Sci. **12**: 99–103. 1910) recorded *D. Willkommii* on fallen twigs of *Larix* in a tamarack bog. In a letter to G. Hamilton Martin, May 28, 1921, Kauffman stated, ". . . I have a collection of what I report as *Dasyscypha Willkommii* Hartig and have never had a critical survey of our tamarack to study its actual effects. I should say off-hand that no serious effect is due to it in the bog swamps of Michigan." Kauffman again reported the fungus from New York State (Bull. N. Y. State Mus. **179**: 80–104. 1915) on twigs of American larch. Again in 1911, Güssow reported *D. Willkommii* Hartig from Nova Scotia (Rep. Dom. Bot. year ending Mar. 1911, p. 259). In a letter to the senior author, June 10, 1931, Güssow commented as follows: "I may say that later evidence revealed that the fungus

reported was not *Dasyscypha Willkommii,* the cause of larch canker, but a related species of no special importance. We have been on the lookout for *D. Willkommii* ever since, but have not found any evidence in Canada."

J. R. Weir in correspondence with A. H. Graves, August 1, 1917, discussed the large-spored *Dasyscypha* forms which he had under observation in the western United States. Quoting in part from his letter he was of the following opinion: "There is absolutely no difference between *Dasyscypha calycina* (Schum.) Fuckel and *D. Willkommii* Hartig. At least, so far as I have ever been able to determine from the examination of material under both names from various parts of Europe and America. . . . The fungus is very common throughout western United States and Canada but I do not consider it of much economic importance. I have found it fruiting in wounds of branches and stems of young trees but it never seems to produce the cankers so much described in European literature. . . . The fungus is usually found fruiting on old fallen twigs and branches. I occasionally find it fruiting on branches of old living mistletoe brooms, which indicates a slow parasitic action. The fact that our American plant does not seem to behave in the same manner as the European plant rather indicates a different form or that we do not have the true *D. Willkommii.* The varieties of *D. calycina,* viz. *Laricis* seems to be nothing more than the true form on different hosts. I find it on *Picea, Larix* and *Abies.*" It is here interesting to note that Sydow and Petrak (Ann. Myc. **20**: 178–218. 1922) later identified *Dasyscypha* specimens on branches of *Larix occidentalis* from Idaho as *D. Willkommii.*

In his manual of economic plant diseases new to or not widely distributed in the United States, J. A. Stevenson (Foreign Plant Diseases, p. 99, U. S. Dept. Agr. 1926) listed the European larch canker parasite *Dasyscypha calycina* (Schum.) Fuckel (*D. Willkommii* Hartig). Stevenson stated that the organism had been reported from Newfoundland and commented upon the possibility of its introduction into the United States. The following hosts were reported: *Larix decidua, L. occidentalis, Abies pectinata, Pinus austriaca, P. laricio, P. pumilio* and *P. sylvestris.* Likewise Seymour in his compilation of published fungus records (Host

Index. 1929), listed *Dasyscypha Willkommii* on *Larix laricina* and *L. occidentalis,* and *D. calycina* (Schum. ex Fries) Fuckel on *Pinus rigida, P. sylvestris* and *Abies balsamea. D. Willkommii* (Hartig) was given as a synonym for the species on pine.

The authors who have examined considerable *Dasyscypha* material collected throughout the United States confirm Kauffman's and Weir's observations for they have never observed typical cankers on native larch species, which were associated with large-spored *Dasyscyphae.* The North American forms on *Larix,* which this study has shown to be distinct morphologically and physiologically from the introduced *D. Willkommii* and *D. calycina,* are described as new species:

Dasyscypha oblongospora sp. nov. (PLATE 11, 12).

Apothecia, waxy, fleshy, sparse (PLATE 11, FIG. 1), scattered or grouped erumpent, at first, globular, closed, opening in a roundish form, margin inclosed, urn-like, becoming expanded under moist conditions laterally compressed and closed when dry (PLATE 11, FIGS. 1, 2), shortly but distinctly stipitate; externally whitish or greyish-white, with long, flexuous excipular hairs minutely roughened, hyaline, thin-walled, cylindrical with subacute, gently rounded extremities (PLATE 12, FIG. 3), septated, forming elongated cells, 3–4 μ broad, persistent; disc salmon-orange to orange-buff, 0.5–2 mm. diameter. Asci clavate, apex obtusely rounded, range (200) $68.4–115.2 \times 7.0–10.0 \mu$; commonly $72–97 \times 7–9 \mu$ (PLATE 12, FIG. 1). Ascospores eight, uniseriate or irregularly and loosely arranged, hyaline, smooth, continuous at first, commonly becoming uniseptate upon germination, distinctly oblong, or oblong-elliptic with obtuse extremities; biguttulate, range (200) $10.0–16.0 \times 3.8–6.0 \mu$, commonly, $11–15 \times 4–6 \mu$ (PLATE 12, FIG. 2). Paraphyses outranking asci, flexuous, septate, filiform not swollen toward the tip or very slightly so, with obtuse extremities, conspicuously minutely guttulate, (100) $90.0–133.2 \times 1.0–2.0 \mu$, extremities 1–2.5 μ broad (PLATE 12, FIG. 1).

Conidial stage consisting of waxy, fleshy, erumpent stromata with irregular, labyrinthiform cavities in which the microconidia (spermatia) are abstricted from the tips of slender, subulate, acutely-pointed, simple or verticillately-branched sporophores (PLATE 12, FIG. 4); microconidia hyaline, continuous, elliptic-oblong or allantoid, with obtuse extremities, (100) $2–5 \times 1–1.5 \mu$ (PLATE 12, FIG. 5). Germination not observed.

Ascomatibus sparsis, solitariis vel gregariis, erumpentibus, distincte breviter stipitatis; initio subglobosis, dein cyathiformibus, cupulis humidulis planiusculis; margine in sicco semper connivente; disco carneo-aurantiaco vel luteo-aurantiaco; 0.5–2 mm. diam.; extus albidis vel griseo-albidis, tomentosis; pilis elongatis, hyalinis, septatis, minute asperatis, 3–4 μ crassis. Ascis octosporis, clavatis subcylindraceis, $68.4–115.2 \times 7.0–10.0\ \mu$, vulgo $72–97 \times 7–9\ \mu$. Ascosporis monostichis, continuis, hyalinis, distincte oblongis, vel oblongis-ellipsoideis $10.0–16.0 \times 3.8–6.0\ \mu$, vulgo $11–15 \times 4–6\ \mu$. Paraphysibus filiformibus, septatis, ascos superantibus, apice obtusis vel rarius sursum incrassatulis, guttulatis, $90.0–133.2 \times 1.0–2.0\ \mu$, apice 1.0–2.5 μ crassis.

Fructificationibus conidicis carnosis, ceraceis, erumpentibus, loculiis simplicis vel labyrinthiformibus, basidiis hyalinis, filiformibus, subuliformibus, simplicibus vel verticillate; ramosis, microconidiis (spermatiis) hyalinis, continuis, ellipsoideo-oblongis, vel allantoidiis, (100) $2–5 \times 1–1.5\ \mu$ crassis.

Hab. in ramulis emortuis *Laricis laricinae, L. leptolepis, L. europeae, Pini pungentis, P. virginianae, Piceae pungentis, Pseudotsugae taxifoliae* in America boreali. Specimen typicum *53156, L. laricina* in Herb. Myc., U. S. D. A., Washington, D. C.

Habit.—The type specimen, *53156* with a slide of *D. oblongospora* collected on dead branches of *Larix laricina,* Bethel, Vt., by Spaulding, May 8, 1931, have been deposited in the Mycological Herbarium, U. S. D. A., Washington, D. C.

Other specimens of the fungus on Abieteae made 1927–1932 in the collections of the Division of Forest Pathology, have been studied:

On *Larix europaea.* Michigan,—*43552,* Fennville, coll. J. R. Hansbrough; *43568,* Bailey, coll. J. R. H.; *53816,* Forest Plantation, Univ. Michigan, coll. D. V. Baxter. Maine,—*16389,* Lisbon Falls, coll. J. R. H.; *53053,* Lisbon Falls, coll. M. A. McKenzie; *53771,* Lisbon Falls, coll. McK. & K. F. Aldrich. Massachusetts,—*43563,* Hamilton, coll. Hahn; *53175,* Hamilton, coll. McK. & K. F. A.; *53191–2,* Hamilton, coll. Hahn & C. K. Goodling; *53745,* Hamilton, coll. McK.; *53749–69–72–73–74,* Nantucket, K. F. A. & McK. New York,—*43549,* Afton, coll. A. G. Baker; *53001,* So. Millbrook, coll. Baker & R. P. True; *53002,* Fulton, coll. Baker. Pennsylvania,—*53767,* Dimock, Baker & True.

On *Larix laricina.* Michigan,—*43577,* Greenville, coll. Baxter. Vermont,—*53039–42–45–47,* Bethel, coll. P. Spaulding, N. O. Howard & Hahn; *53156,* Bethel, coll. P. S. New Hampshire,—*53074,* Oxford, Cube Mt., C. S. Moses; *53872,* Littleton, J. R. H. Massachusetts,—*16212,* Ipswich, J. R. H.; *53021,* Richmond, Baker; *53044,* Hamilton, N. O. H.; *53720,* Ipswich, McK. & K.

F. A. Connecticut,—*53000,* Sharon, Baker; *53810,* Sharon, J. L. Bedwell. New York,—*16369,* Woodgate, H. Metcalf; *43574,* Boonville, Baker; *53020,* Nundara, Baker, *53023,* Saratoga, Baker; *53024,* Millerton, Baker; *53078,* Loon Lake, Baker. Pennsylvania,—*53759–60–62–63–64–65–93,* Pocono Mts., Baker & True; *53766,* Carbondale, Baker & True; *53768,* Thompson, Baker & True, *53770,* Dimock, Baker & True. Quebec Prov.,—*63198,* Bonaventure, Eno.

On *Larix leptolepis.* Massachusetts,—*43560,* Hamilton, H. Metcalf, P. S., N. O. H. & Hahn; *43598,* Ipswich, coll. N. O. H., J. R. H. & Hahn; *43599,* Hamilton, coll. N. O. H., J. R. H. & Hahn.

On *Picea pungens* Engel. Massachusetts,—*53755,* Hamilton, coll. Ayers; *53839,* Hamilton, coll. Hahn.

On *Pinus pungens* Lam. Pennsylvania,—*53881,* Greenwood Furnace, coll. P. S.

On *P. virginiana* Miller. Pennsylvania,—*16717,* Stone Creek, coll. L. O. Overholts & P. S.

On *Pseudotsuga taxifolia* (blue form). Massachusetts,—*16201,* Hamilton, coll. J. R. H.; *53185,* Hamilton, coll. Goodling & Hahn.

Dasyscypha occidentalis sp. nov. (PLATE 12, 13).

Apothecia waxy, fleshy, abundant (PLATE 13, FIGS. 1, 2, 3), scattered or grouped, erumpent, at first globular, closed, opening in a roundish form, margin inclosed, urn-like, becoming widely expanded, saucer-like under moist conditions (PLATE 13, FIG. 3), laterally compressed and closed when dry; shortly but distinctly stipitate; externally whitish with flexuous, cylindrical hairs minutely roughened, hyaline, thin-walled with blunt, obtuse, gently rounded extremities, septated, forming short cells, 3–4 μ broad, brittle, readily breaking away revealing the glabrous ascocarp beneath (PLATE 12, FIG. 8); disc orange-buff to salmon-orange, 1–3 mm. diameter. Asci clavate, apex obtusely rounded, range (400) 86.4–129.6 $\times$ 6.0–12.0 μ, commonly, 94–119 $\times$ 8–10 μ (PLATE 12, FIG. 6). Ascospores eight uniseriate, arranged in a close-set, regular, oblique manner, hyaline, smooth, continuous at first, becoming commonly uniseptate on germination, elliptical, blunt with obtuse extremities, range (380) 10.6–18.0 $\times$ 4.0–7.8 μ, commonly, 12–16 $\times$ 5–6 μ (PLATE 12, FIG. 7). Paraphyses outranking asci, flexuous, filiform, septate, distinctly swollen at tips, becoming

spathulate, interspersed with paraphyses unswollen at the tips or just slightly so, (222) 82.8–180.0 × 1.0–2.0 μ, extremities 1–5 μ broad (PLATE 12, FIG. 6).

Conidial stage consisting of a waxy, fleshy, erumpent stromata with irregular labyrinthiform cavities in which the microconidia (spermatia) are abstricted from the tips of slender pointed or simple verticillately branched sporophores (PLATE 12, FIG. 9); microconidia continuous, elliptic-oblong or allantoid with obtuse extremities, (100) 2–5 × 1–1.5 μ (PLATE 12, FIG. 10). Germination not observed.

Ascomatibus confertis, solitariis vel gregariis, erumpentibus, distincte breviter stipitatis, initio subglobosis, dein cyathiformibus, cupulis humidulis expansis; margine in sicco incurvatis; disco carneo-aurantiaco, 1–3 mm. diam.; extus primitus tomentosis, albidis, demum nudis, glabris; pilis, hyalinis, septatis, minute asperatis, 3–4 μ crassis. Ascis octosporis, clavatis, subcylindraceis, 86.4–129.6 × 6.0–12.0, vulgo 94–119 × 8–10 μ. Ascosporis monostichis, continuis, hyalinis, ellipsoideis, ellipsoideo-oblongis vel ovatis, apice obtusis, 10.6–18.0 × 4.0–7.8 μ, vulgo 12–16 × 5–6 μ. Paraphysibus filiformibus, septatis, ascos superantibus, apice obtusis, vulgo distincte sursum incrassatulis, spathulatis, vel raro submoniliformibus, 82.8–180.0 × 1.0–2.0 μ; apice 1–5 μ crassis.

Fructificationibus conidicis carnosis, ceraceis, erumpentibus, loculiis simplicis, vel labyrinthiformibus; basidiis, hyalinis, filiformibus, subuliformibus, simplicibus vel verticillate ramosis; microconidiis (spermatiis) hyalinis, continuis, ellipsoideo-oblongis vel allantoidiis, 2–5 × 1–1.5 μ crassis.

Hab. in ramulis emortuis *Laricis occidentalis, L. europaea, L. leptolepis, L. laricinae* in America boreali. Specimen typicum *40485, L. occidentalis,* in Herb. Myc. U. S. D. A., Washington, D. C.

Habit.—The type specimen, *40485,* with a slide of *D. occidentalis,* collected on dead branches of *Larix occidentalis,* Hills, B. C., by J. L. Mielke, June 23, 1928, have been deposited in the Mycological herbarium, U. S. D. A., Washington, D. C.

Other specimens of the fungus on Abieteae, made in 1927–1928 and 1931, in the collections of the Division of Forest Pathology, have been studied:

On *Larix europaea.* Massachusetts,—*53174,* Hamilton, coll. McKenzie & K. F. Aldrich. New York,—*43548,* Woodgate, coll. Baker; *53003,* Warrensburg, coll. Baker.

On *Larix laricina.* Vermont,—*16271,* Bethel, coll. P. Spaulding; *53038–40–157,* Bethel, coll. P. S., N. O. Howard & G. G. Hahn. New York,—*43573,* Utica, coll. Baker; *53022,* Brook, coll. Baker; *53077–86,* road from Johnstown to Ephrata, coll.

Baker & R. P. True. Pennsylvania,—*53770,* Dimock, Baker & True.

On *Larix leptolepis.* Massachusetts,—*53041–073,* Hamilton, coll. Hahn; *53075,* Hamilton, coll. Hahn, T. T. Ayers & C. S. Moses.

On *Larix occidentalis.* Washington,—*52372,* Northport, coll. G. G. Hedgcock. Oregon,—*40693,* Horse Thief Meadows, Mt. Hood, coll. J. R. Hansbrough. Montana,—*16270,* Belton, coll. C. R. Stillinger. British Columbia,—*40473* (dupl. *45825*), Nelson, coll. J. L. Mielke; *40484,* Hunters Siding (Rosebery) coll. J. L. M.; *40485,* Hills, Slocan Lake, coll. J. L. M. & H. G. Lachmund; *40512,* Hunters Siding, coll. J. R. H.; *45824,* Hills, coll. H. G. L.; *53100,* Hunters Siding, coll. J. R. H.; *53101,* Apex (Nelson), coll. J. R. H.

CULTURE NOTES

There is not available space in this article to give in detail all the culture data with respect to the Dasyscypha group herein discussed. In all approximately 2,000 cultures have been studied which have aided greatly in allocating forms. Only the following brief notes will be given at this time. Mono-ascus and mono-ascospore cultures of the *Dasyscypha* species grew slowly but vigorously upon 3 per cent malt agar. In the earlier studies a malt produced in Brunswick, Germany, which had been used with success by Plassmann (10), was employed. It was found that culture growth succeeded best at low temperatures and on substrata having a pH value approximately 5.0 to 6.0. The ascospores which germinated in certain instances after nine months' keeping were commonly uniseptate with polar germ tubes. Upon the malt medium the following characteristics were recorded:

Dasyscypha calycina. Germinated ascospores produced an elongate, scantily branched, flexuous type of germination (PLATE 8, FIG. 3) within three days. Strains of the fungus produced a whitish, close-set, low, "mealy," aërial growth (PLATE 8, FIG. 4) over the surface of the slant which showed indistinct zonations and a somewhat ray-like or striated appearance. A dense, white-tufted, cottony growth formed at the apex of the slant and a light pinkish-buff or ochraceous-buff, or cinnamon-buff color formed in the

aërial hyphal stratum. Cultural agreement between American and Scottish cultures was observed. The imperfect stage formed scantily.

D. Willkommii. Germinated ascospore produced a clumped, " mycorrhiza-like " germination consisting of profusely-branched, comparatively short hyphae, pronouncedly sinuous or " curly " in appearance (PLATE 10, FIG. 5). Lohman (Mich. Acad. Sci. **15**: *pl. 11, fig. 4,* 1932) figures a somewhat similar branched, coiling type of germ tube development for *Hysterographium fraxini* (Fries) de Not. Strains of the fungus isolated from cankers in Massachusetts (PLATE 10, FIG. 1) showed extremely little variation and agreed culturally with those isolated in Scotland. A dense, chalky-white, velvety, aërial growth covered the slant with evident zonations (PLATE 10, FIG. 4). Dense, cottony mycelial tufts, some 5 mm. across at the top, formed at the apex of the slant and along the sides of the tubes. A light pinkish-buff or pale ochraceous-buff color appeared in the aërial hyphae which became ochraceous-salmon with age. The imperfect stage formed scantily.

D. oblongspora. Germinated ascospores produced elongate germ tubes which in three days were unbranched or had produced exceedingly few, short lateral branches (PLATE 11, FIG. 3). This species produced the imperfect stage in great abundance. The surface of the slant was covered with a low-growing, whitish, coarse-matted, woolly, indistinctly zonate, aërial growth, somewhat flocculent in appearance in which formed ochraceous-tawny, conidial fruit bodies. With age a light buff color appeared and the conidial exudates became Mars brown. Tufted hyphae were lacking at the apex of the slant (PLATE 11, FIG. 4).

D. occidentalis. Germinated ascospores produced a clumped, branched germination within 3 days, comparable with that produced by *D. Willkommii* except that the hyphae instead of being " curly," were flexuous, tending to be straight (PLATE 13, FIG. 5). The colony was completely covered by a dense, fine, woolly growth, slightly zonate, which became tinged with a light buff color (PLATE 13, FIG. 4). A tawny color appeared in the substratum. The imperfect stage was produced scantily.

On a natural medium consisting of fresh twigs of European larch sterilized with a plug of ground oat agar, the imperfect stage formed more readily with all the species. However, it was very noticeable in the case of *D. oblongospora* that microconidia production was most abundant.

DISCUSSION

The data dealing with the taxonomy and nomenclature of the large-spored *Dasyscypha* forms presented in this paper, will be of value not only to mycologists but pathologists as well. Our problem has been to attempt to say with accuracy what is meant by *Dasyscypha calycina* (Schum.) Fuckel and *D. Willkommii* (Hartig) of the European writers. Heretofore these names have been interpreted so variously that the actual occurrence of these species in North America has been seriously open to question. It is very probable that many of the American collections, heretofore identified as *D. calycina* or *D. Willkommii,* are the two new species herein described, or separate species. This whole matter of nomenclature would be of purely academic interest but for the fact that the host range of the European larch canker parasite involves economically important species.

In Europe it is the general consensus of opinion that the larch canker organism occurs parasitically and saprophytically on a number of coniferous hosts including Douglas fir. At the beginning of this investigation, morphological and physiological differences became evident to the senior author while conducting a preliminary investigation of larch and Douglas fir *Dasyscypha* forms in Scotland in 1929. Culture studies were first made of the saprophytic form on green Douglas fir growing intermixed with badly diseased European larch in a plantation at Glentress, Peeblesshire, Scotland. This *Dasyscypha* with smaller apothecia occurring on the Douglas fir, fruited only sparsely upon the lower suppressed branches, which were dead or dying. Typical *Dasyscypha* cankers, which occurred so abundantly on the larch, were not present on the Douglas fir. Isolations of single ascospores from the Douglas fir *Dasyscypha,* produced a type of germination (PLATE 8, FIG. 3) distinct from that formed by isolations made from the large fruit bodies of *D. Willkommii* taken from active

larch cankers on living tissue (PLATE 10, FIG. 5). The results of this test were repeated several times.

The same phenomenon was sought among germinated ascospores taken from ascocarps produced on the active canker itself and from smaller fruit bodies produced upon dying and dead larch trees, and upon fallen branches. Isolations of ascospores from this smaller form produced a type of germination (PLATE 8, FIG. 3) identical with that produced by the *Dasyscypha* form on Douglas fir. Cultures from this source (PLATE 8, FIG. 4) proved to be distinctly different from those of *D. Willkommii* (PLATE 10, FIG. 4).

These two forms were observed fruiting upon the same cankers on small living branches of the European larch. One of these which appeared to be the parasite, produced abundantly, large. robust apothecia (PLATE 10, FIGS. 2, 3), whereas the other (PLATE 8, FIG. 2), confined to the tissue weakened by the girdling action of the canker, fruited sparsely. Later, however, after the death of the branch part had occurred, this secondary form produced abundant apothecia. Isolations from these two forms produced the distinct types of germination and culture characteristics alluded to above. The secondary or saprophytic form was later determined as *D. calycina.*

Subsequent isolation studies of the introduced larch canker organism in the United States, have shown the same culture characters as those previously observed in Scotland. This can also be said for the European saprophytic species, *D. calycina,* which was probably introduced into New England simultaneously with *D. Willkommii;* for it has only been found in the area where *D. Willkommii* was introduced.

It is the opinion of the writers that the European larch canker parasite does not occur on hosts other than those belonging to the genus *Larix,* and always in close association with the lesions produced by the fungus. When the part attacked has been killed or weakened, secondary saprophytic forms come in. Artificial inoculation studies have shown that whereas *D. Willkommii* will infect larch species, the organism will not grow upon inoculated weakened larch, dead or dying, or upon living or weakened Douglas fir (blue form). The native *Dasyscyphae* described in this

paper are known only as saprophytes, and *D. calycina* is regarded likewise. *D. calycina* would not infect healthy inoculated larch or Douglas fir, but when inoculated into plants of the latter host, which were dying, the fungus produced fruiting bodies.

Since *D. Willkommii* has been found only on species of larch, Plassmann (**11**) is quite correct in his opinion that this fungus is not to be feared as a new enemy of Douglas fir. However, it is the author's belief that Plassmann was concerned with *D. calycina,* or a nearly related form, when he confirmed Day (Empire For. Jour. **7**: 112–115, 1928) in his determination of British material of the European larch canker parasite on *Pinus sylvestris* and *Pseudotsuga taxifolia* (green form). Although it must be admitted that Plassmann questioned the parasitism of the organism on Douglas fir, he tended to regard *D. Willkommii* as a saprophyte on that host in Britain.

The two types of germination discussed for *D. Willkommii* and *D. calycina* are distinctly different from those of the native forms. Because the latter differ morphologically as well as physiologically they are tentatively regarded as new species. Because of the close natural relationships between the species of this group, segregation proved somewhat difficult, *i.e.,* the separation of *D. calycina* and *D. occidentalis.* Nevertheless the authors regard these two physiologically distinct forms as separate species, not only because of the larger size of the asci and spores of *D. calycina* but also because of the submoniliform type of paraphyses (PLATE 9, FIG. 6) produced commonly by the fungus. The latter structures while not entirely lacking in *D. occidentalis* are replaced in the new species by filiform paraphyses characteristically more or less swollen at the tips (PLATE 12, FIG. 6). Further differentiation among the large-spore forms was sought in a comparison of the imperfect stages, but sufficient difference was not found.

Because of the close agreement between cultures of *D. Willkommii,* the authors regard this as a homogenous species. They consider the production of large apothecia and large ascospores as an inherent character, rather than the result of the growth of the fungus on different substrata. On the other hand, Hiley (**6**, p. 79) and other European workers concluded that the European larch canker organism was a heterogenous species, comprised of

a number of intermediate forms between the parasitic and saprophytic types. In Europe, as in America, a number of closely related forms may be present which can be differentiated only by an intensive morphological and physiological study.

SUMMARY

With the discovery in 1927 of the European larch canker disease in Massachusetts, a study has been made of the organisms associated with the canker in order to determine their relationships. The identification of the causal fungus and its distribution in the United States became a problem of considerable importance because of the economic host species involved; for in Europe the European larch canker organism has been reported on the valuable Douglas fir and other conifers. Furthermore fungi purported to be the European larch canker parasite were recorded as being present in North America, before the actual discovery of the disease in this country.

The organism which has been demonstrated as causing European larch canker, should be called *Dasyscypha Willkommii* (Hartig) Rehm, and not *D. calycina* (Schum.) Fuckel. The introduced parasite (*D. Willkommii*) was identified on imported *Larix europaea* and *L. leptolepis* in two localities in Massachusetts. The organism was always found in close association with the cankers which it produced on living tissue. Although the blue form of Douglas fir (*Pseudotsuga taxifolia*) was growing in close proximity to the diseased *Larix europaea,* it did not become infected with the European larch canker disease. Artificial infection with *D. Willkommii* succeeded on young trees of species of larch, but they were unsuccessful not only on dead or dying tissue of the same host, but also upon healthy and dying Douglas fir (blue form).

Dasyscypha calycina Fuckel (nec *Peziza calycina* Schum.), as has been pointed out in this paper, is distinct morphologically and physiologically from *D. Willkommii,* and should be recognized as separate species. It should not be confused with the pre-Friesian species, *P. calycina* Schum. or with *P. calycina* Fr. An amended description is given.

D. calycina which is regarded as a saprophyte, is believed to have been introduced into this country with the European larch canker parasite, for it has only been collected in the area where the European disease was discovered. *D. calycina* was collected on *Larix europaea, L. leptolepis* and *Pseudotsuga taxifolia.* Artificial inoculations with *D. calycina* on living larch and Douglas fir (blue form) did not succeed. The fungus was able, however, to produce fruiting bodies on the same form of Douglas fir which was dying at the time of inoculation, thereby showing its saprophytic nature.

Both *D. Willkommii* and *D. calycina* are distinct morphologically and physiologically from nearly related, large-spore, native forms occurring on larch and other coniferous species. These forms, which heretofore have been identified as *D. Willkommii* or *D. calycina,* are described (p. 88, 90) as new species: *D. oblongospora* Hahn and Ayers, collected on *Larix laricina, L. europaea, L. leptolepis, Pseudotsuga taxifolia,* blue form, *Picea pungens, Pinus pungens* and *P. virginiana; D. occidentalis* Hahn and Ayers, collected on *L. occidentalis, L. laricina* and *L. leptolepis.* These native organisms, the present known geographical range of which is given, do not cause canker on larch or Douglas fir.

Grateful acknowledgment is due Dr. Perley Spaulding, Division of Forest Pathology, for rendering every possible assistance in obtaining *Dasyscypha* material for study and the opportunity of using his extensive bibliography file; and to Dr. W. W. Diehl, Division Mycology, Bureau of Plant Industry, for valuable information with regard to herbarium specimens and advice with respect to matters of nomenclature and taxonomy. The writers would also tender thanks to the following who have kindly loaned or given information concerning specimens: Director R. Chodat, Herbier Boissier, Geneva; Prof. Dr. K. von Tubeuf, Botanisches Institut, Munich; Prof. Dr. E. Ulbrich, Botanisches Museum, Universität Berlin; Dr. Eberhard Plassmann, Forstlichen Hochschule, Hann-Münden; Director A. W. Hill and Miss E. M. Wakefield, Royal Botanic Gardens, Kew; Dr. Malcolm Wilson, University of Edinburgh, Royal Botanic Garden; the late Mr. A. B. Seymour, the officials of Farlow Herbarium, Harvard University and Dr.

C. W. Dodge, formerly of that institution; the Director and Dr. F. J. Seaver of The N. Y. Botanical Garden. We are also grateful to Dr. G. D. Darker for fresh European material and to Mr. J. R. Hansbrough, Division of Forest Pathology, not only for assistance in the early Dasyscypha culture study but also for numerous collections that were augmented by various workers in the same Division. We are indebted to Mr. M. L. F. Foubert for a number of the photographs of the Dasyscypha habit studies.

DIVISION OF FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY,
IN COÖPERATION WITH THE OSBORN BOTANICAL LABORATORY,
YALE UNIVERSITY, NEW HAVEN, CONN.

EXPLANATION OF PLATES

PLATE 8

Dasyscypha calycina Fuckel (nec *Peziza calycina* Schum.).

Fig. 1. Type Fungi Rhen. *1206,* Herb. Fuckel, Herbier Boissier, Institut Botanique, Geneva. The figure shows only a portion of the entire specimen photographed. Approx. × 6.

Fig. 2. Habit, on *Larix europaea.* Approx. × 11.5

Fig. 3. Ascospore on surface of malt agar, three days after germination. × 300.

Fig. 4. Two four-months-old mono-ascospore malt agar cultures isolated from a single ascocarp, coll. *43564,* saprophytic, *Larix europaea,* Hamilton, Mass. Note: "mealy" aërial growth. Nat. size.

PLATE 9

All drawings made with camera lucida, × 750. Those of the imperfect stage were made from material procured in mono-ascospore culture.

Dasyscypha Willkommii (Hartig) Rehm.

Fig. 1. Asci and paraphyses.
Fig. 2. Ascospores.
Fig. 3. Excipular hairs.
Fig. 4. Sporophores.
Fig. 5. Microconidia.

Dasyscypha calycina Fuckel.

Fig. 6. Asci and paraphyses.
Fig. 7. Ascospores.
Fig. 8. Excipular hairs.
Fig. 9. Sporophores.
Fig. 10. Microconidia.

PLATE 10

Dasyscypha Willkommii (Hartig) Rehm.

Fig. 1. Typical European larch canker on *Larix europaea,* Hamilton, Mass., showing apothecia fruiting on lesion of branch killed by the fungus. Approx. nat. size.

Fig. 2. Apothecia not fully expanded. Note: heavy chalky-white rim. Approx. × 11½.

Fig. 3. Apothecia showing the robust convolute margin. Under moist conditions this may split in a cruciform manner. Approx. × 11½.

Fig. 4. Two three-months-old monoascospore cultures on malt agar, isolated from a single ascocarp, coll. *43559,* Hamilton, Mass., from a canker on a living branch of *Larix europaea.* Note: dense white, velvety aërial growth. Nat. size.

Fig. 5. Ascospore three days after germination on malt agar. Note: "curly" or "mycorrhizal" type of mycelial growth. × 300.

PLATE 11

Dasyscypha oblongospora Hahn & Ayers.

Fig. 1. Habit, on *Larix europaea,* material, coll. *53053,* Lisbon Falls, Maine. Approx. × 11½.

Fig. 2. Apothecia on *Picea pungens,* scant material. Culturally the spruce form agreed with the strains isolated from larch. Approx. × 11½.

Fig. 3. Ascospore from material collected on *Larix laricina,* Bethel, Vt., three days after germination on malt agar. × 300.

Fig. 4. Two two-and-one-half-months-old mono-ascus cultures on malt agar. Isolations were made from a single ascocarp, coll. *43560,* dead twigs of *Larix europaea.* Nat. size.

PLATE 12

All drawings made with the camera lucida × 750. Those of the imperfect stage were made from material procured in mono-ascospore culture.

Dasyscypha oblongospora Hahn & Ayers.

Fig. 1. Asci and paraphyses.
Fig. 2. Ascospores.
Fig. 3. Excipular hairs.
Fig. 4. Sporophores.
Fig. 5. Microconidia.

Dasyscypha occidentalis Hahn & Ayers.

Fig. 6. Asci and paraphyses.
Fig. 7. Ascospores.
Fig. 8. Excipular hairs.
Fig. 9. Sporophores.
Fig. 10. Microconidia.

PLATE 13

Dasyscypha occidentalis Hahn & Ayers.

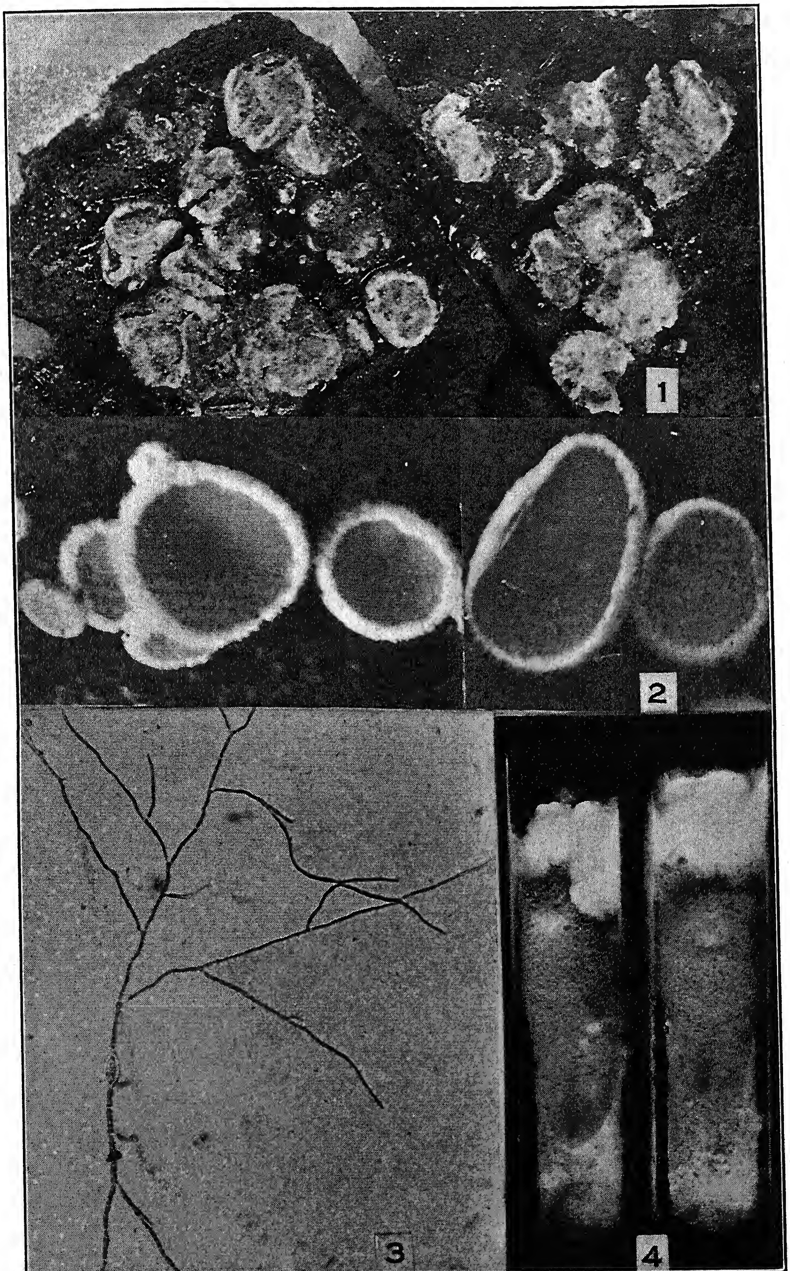
Fig. 1. Apothecia, British Columbia material, coll. *40512,* Hunters Siding, Rosebery, saprophytic on dead twigs, *Larix occidentalis.* Approx. × 11½.

Fig. 2. Habit, Bethel, Vt., material, coll. *53038,* saprophytic on *L. laricina.* Approx. × 4.

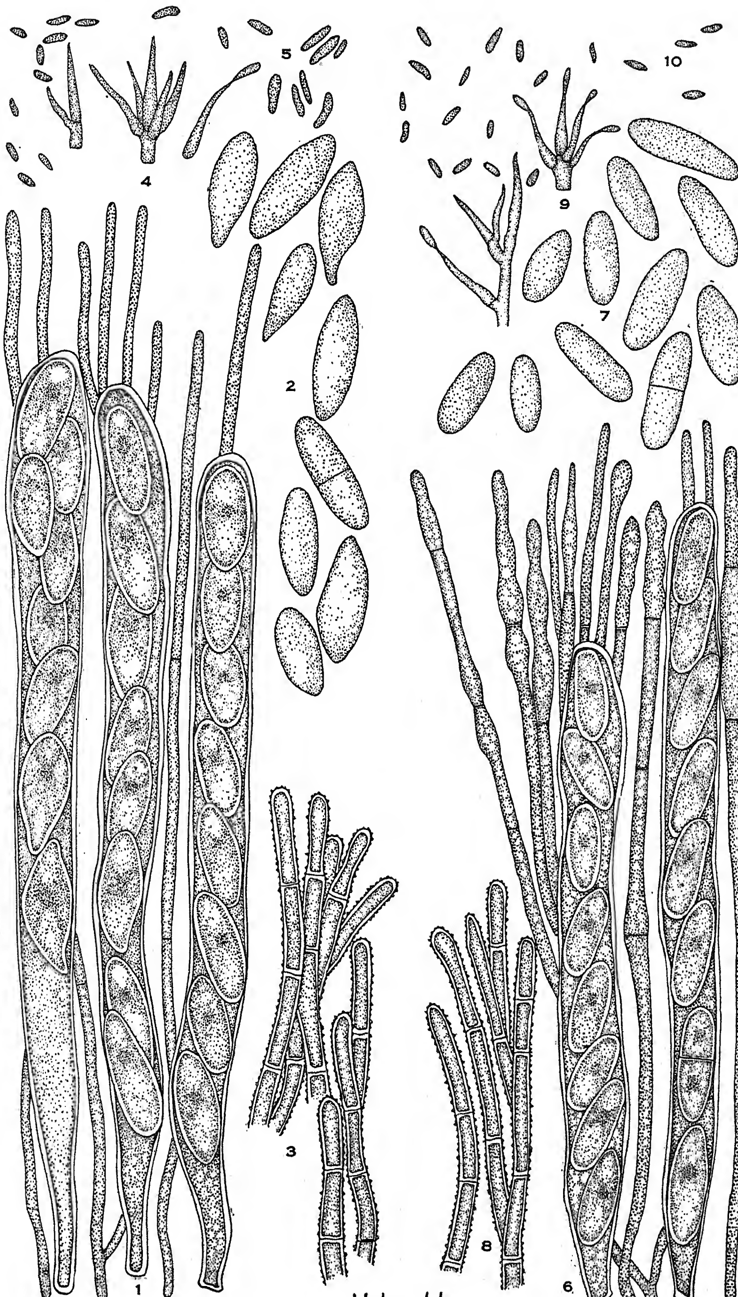
Fig. 3. Habit, Hamilton, Mass., material, coll. *53087,* saprophytic on *L. leptolepis.* Cups moist and expanded. Approx. × 4.

Fig. 4. Three two-months-old mono-ascospore cultures on malt agar; one on left from *L. occidentalis,* B.C., two on right from *L. laricina,* N. Y. Note: dense aerial growth covering entire colony. Nat. size.

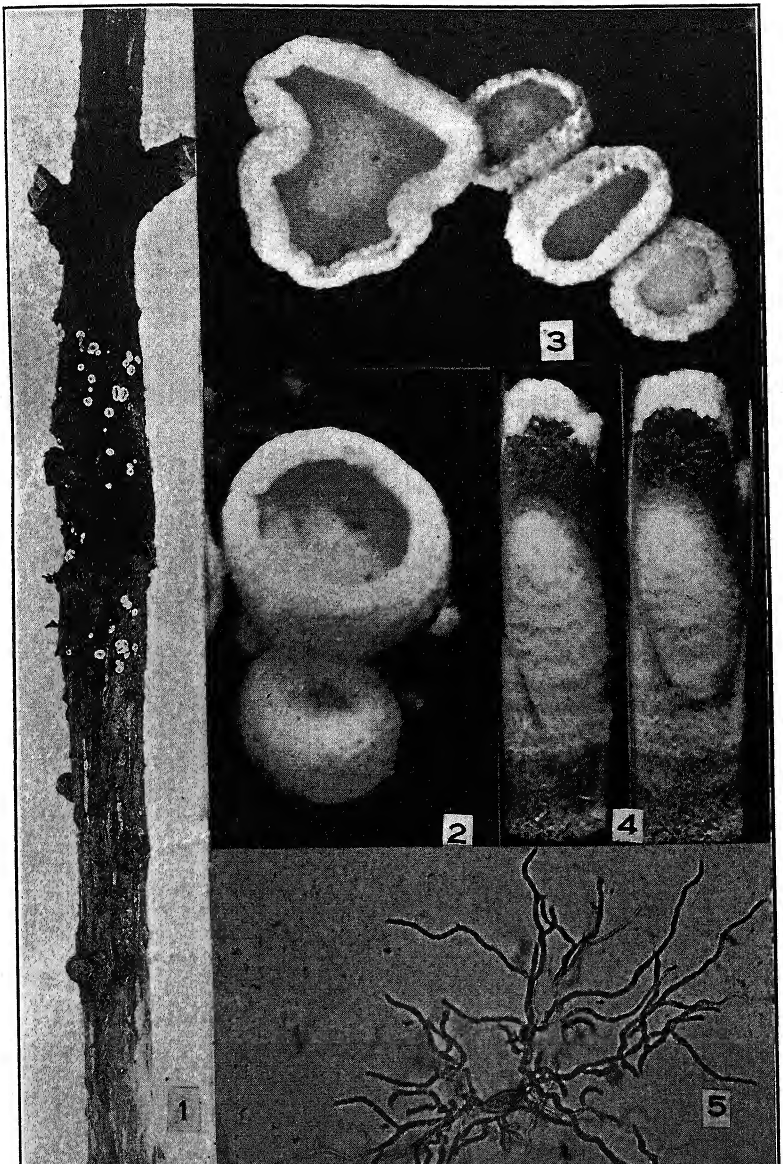
Fig. 5. Germinated ascospore (N. Y. material) four days old on malt agar. Note: clumped type of growth but not "curly" as in the case of *D. Willkommii.* × 300.



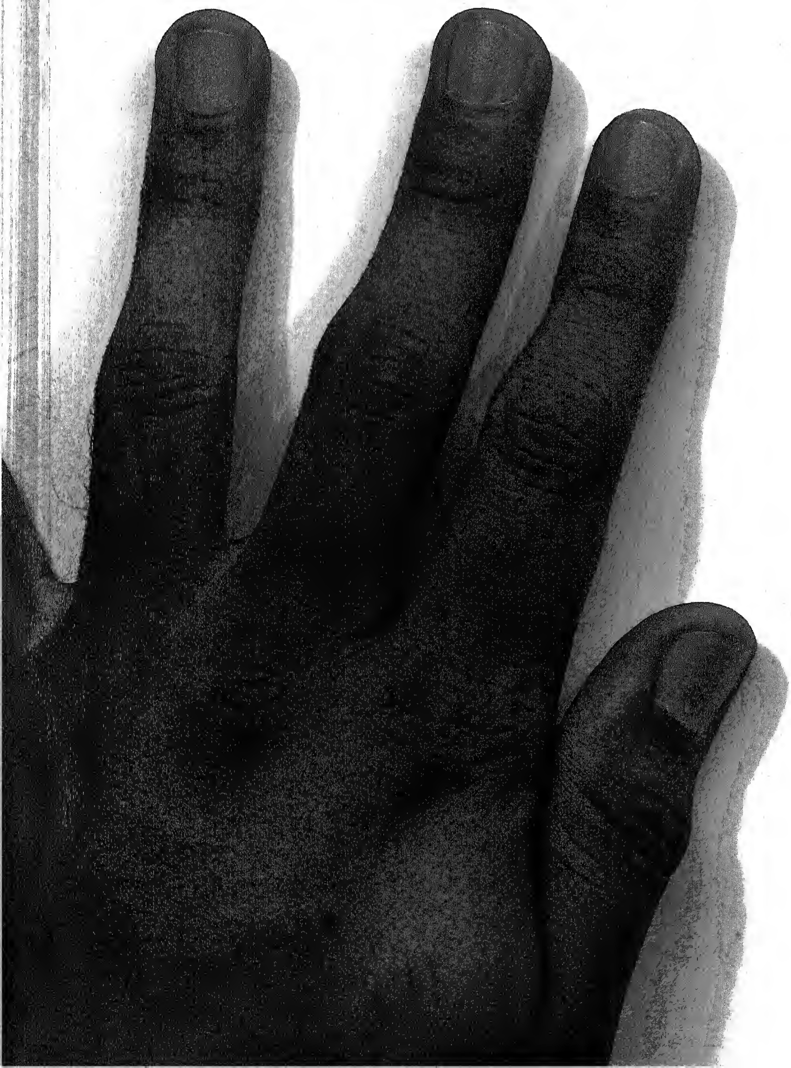
Dasyscypha calycina

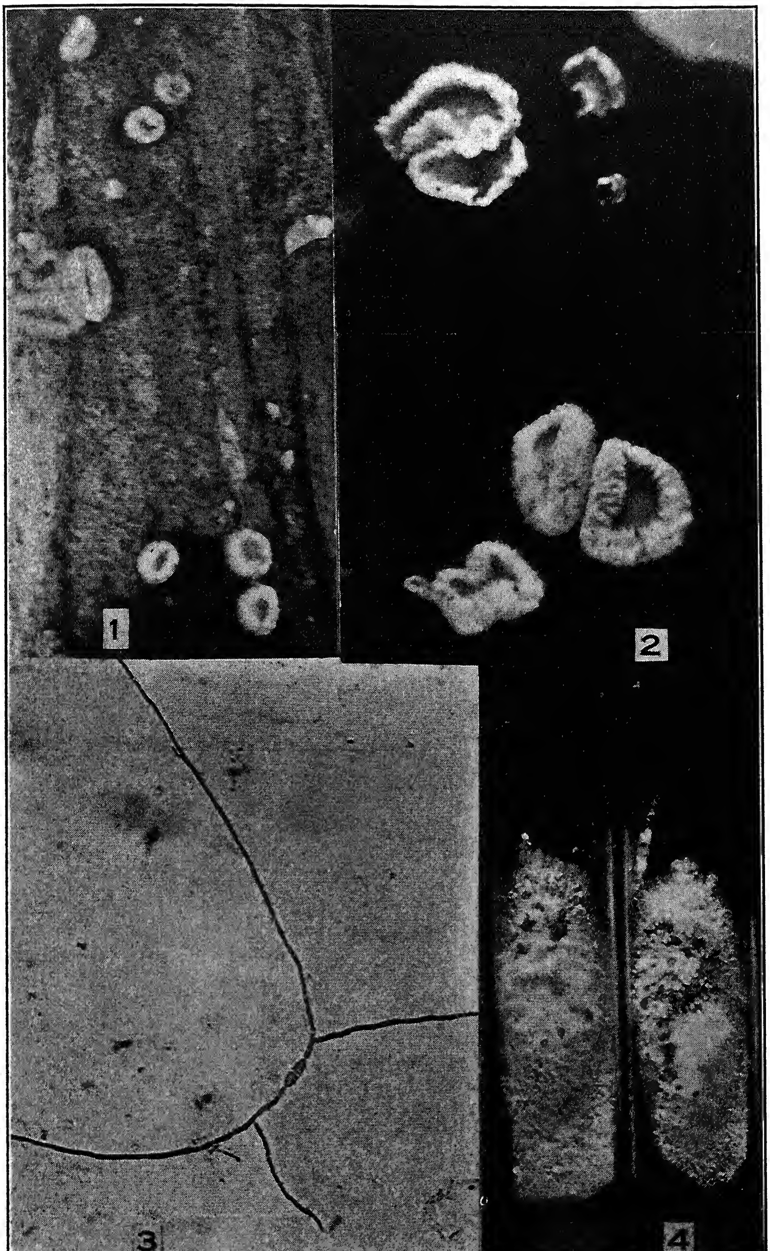


1–5 DASYSCYPHA WILLKOMMII
6–10 DASYSCYPHA CALYCINA

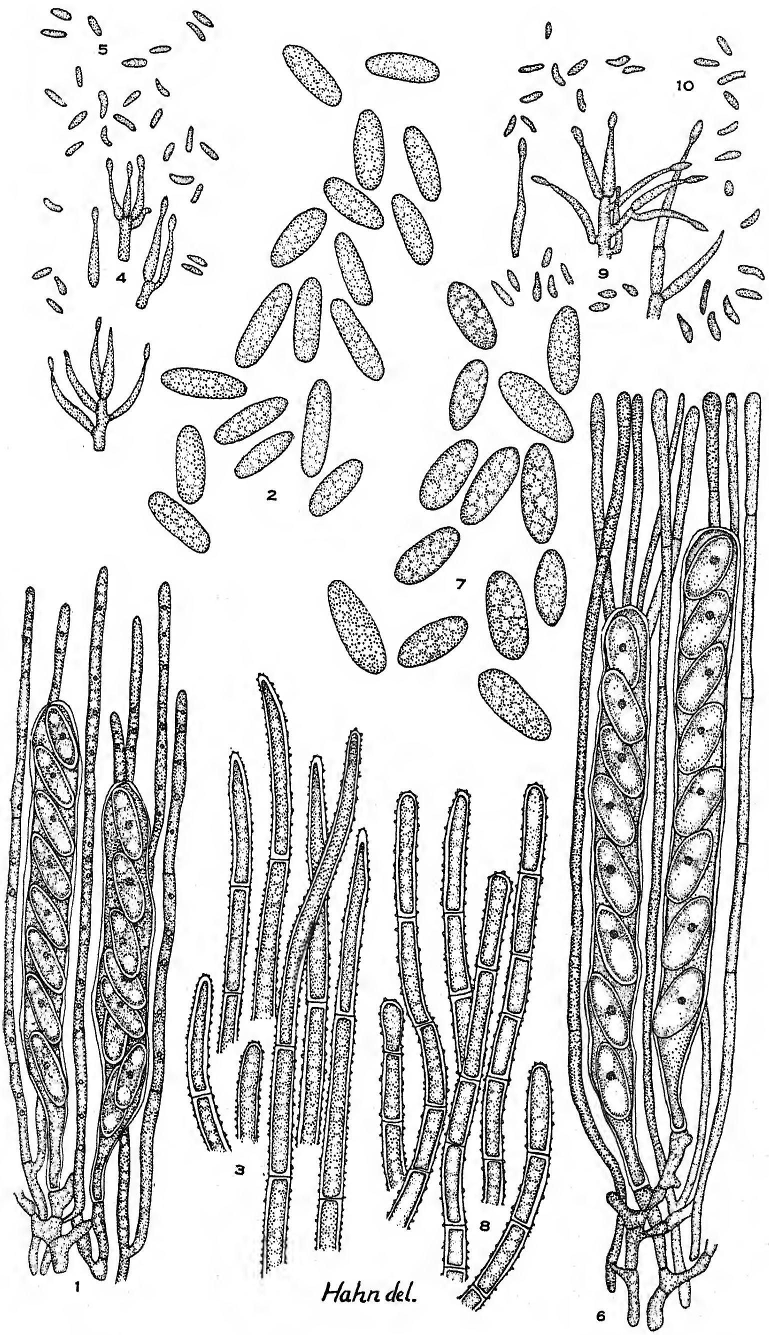


Dasyscypha willkommii

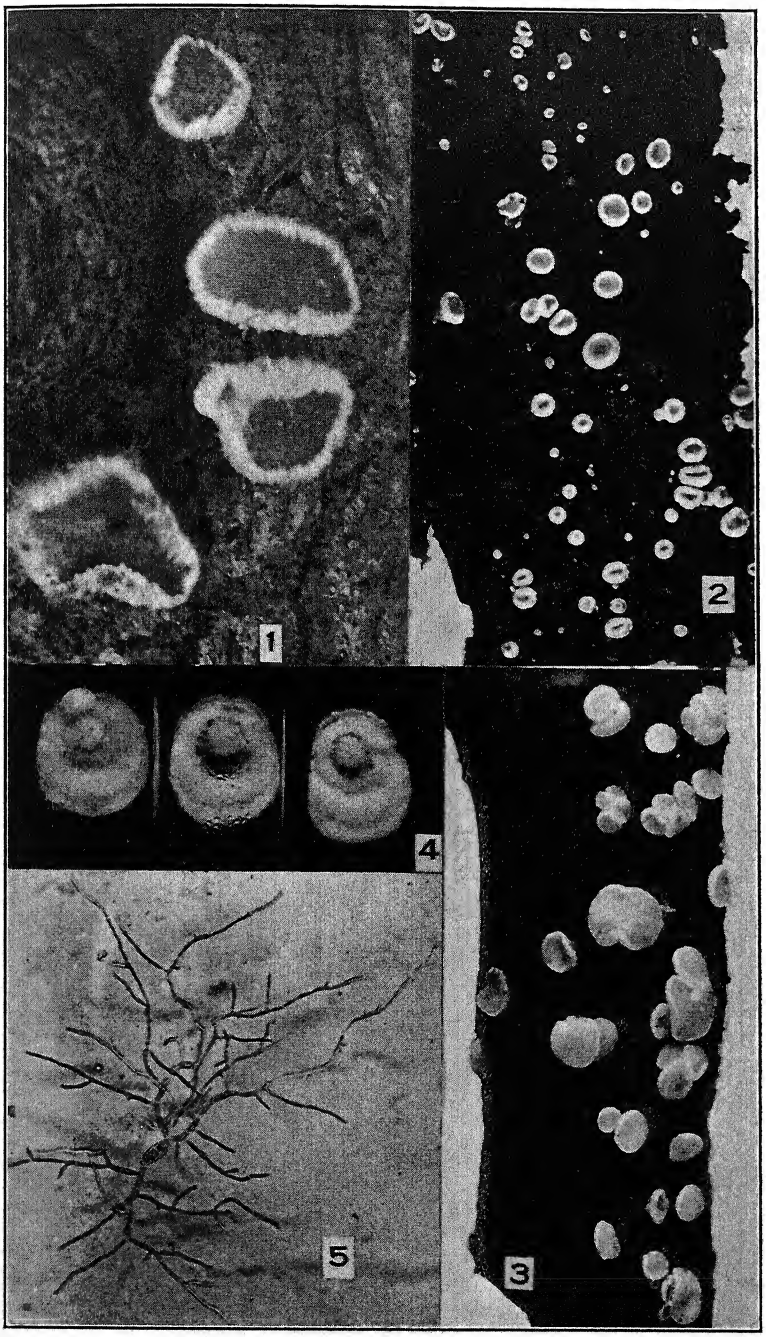




DASYSCYPHA OBLONGOSPORA



1–5 DASYSCYPHA OBLONGATA
6–10 DASYSCYPHA OCCIDENTALIS



DASYSCYPHA OCCIDENTALIS

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PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI—XX. A NEW LAMPROSPORA

Fred J. Seaver

(with plate 14)

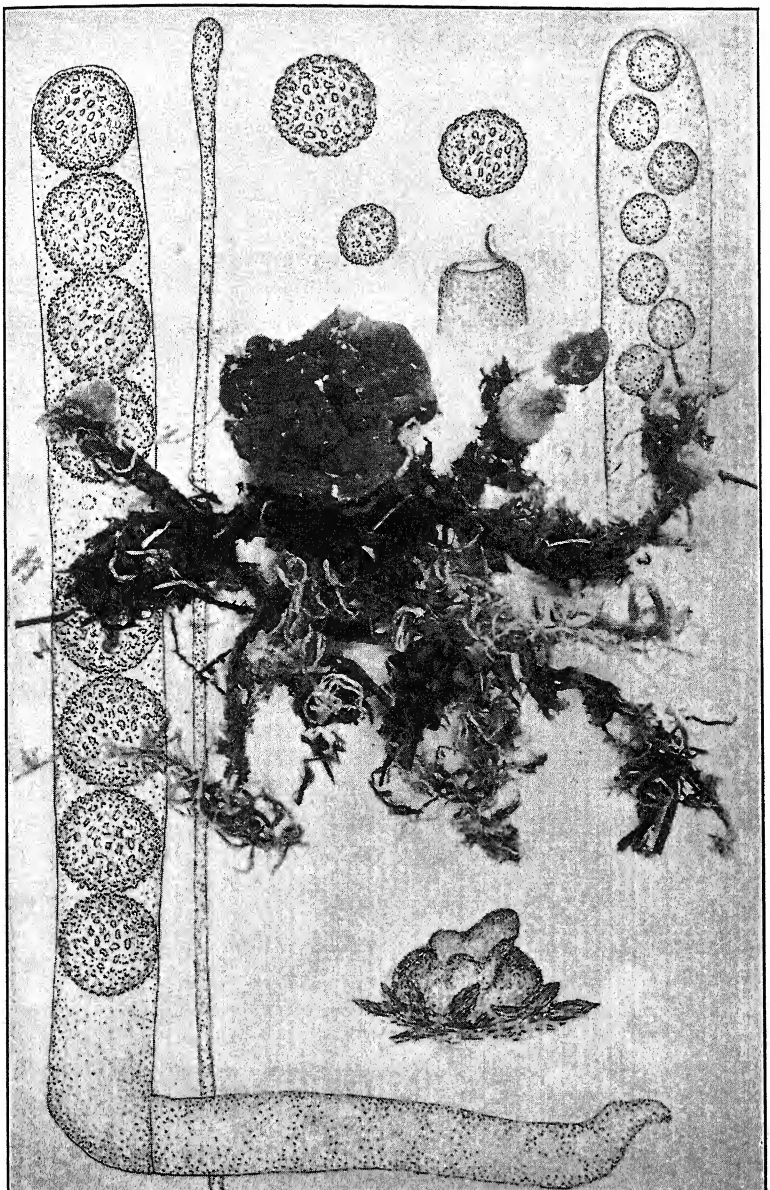
During the summer of 1932 the writer received a collection of cup-fungi growing on *Sphagnum* which had probably been sterilized. Examination at once revealed the fact that it was a species of *Lamprospora* of unusually large dimensions. The spores of this species are quite similar to those of *Lamprospora trachycarpa* but the apothecial characters are entirely different.

Several young apothecia were present and consisted of a globose solid ball reaching nearly a centimeter in diameter and partially surrounded by the leaves and stems of *Sphagnum* on which it was growing. As the apothecia mature the hymenium gradually expands in an irregular manner giving rise to a much convoluted surface. The entire growth is at first whitish later assuming a lavender tint. The one mature apothecium reached a diameter of 3 centimeters.

This species is so different from any which the writer has examined in his studies on this group that he feels compelled to describe it as new. The technical description is as follows:

Lamprospora sphagnicola sp. nov.

Apothecia sessile at first globose and solid, reaching a diameter of 1 cm., gradually expanding and becoming subdiscoid finally reaching a diameter of 3 cm., externally whitish; hymenium strongly convoluted, at first light colored gradually assuming a lavender tint; asci cylindric or subcylindric, tapering into a rather abrupt stem-like base, 8-spored, reaching a length of 215 μ and a diameter of 15 μ; spores at first irregularly disposed, finally becoming definitely 1-seriate, hyaline or subhyaline, at first smooth, soon becoming sculptured reaching a diameter of 12 to 15 μ; spore-sculpturing taking the form of tubercles or very short elongated ridges; paraphyses filiform rather strongly enlarged above reaching a diameter of 4 to 5 μ.



LAMPROSPORA SPHAGNICOLA

Apothecia sessilia, primum globosa dein expansa, maturitate hymenio convoluto; ascis cylindraceis v. subcylindraceis, 200 μ long. et 12–15 μ diam.; sporis globosis, primum levibus dein tuberculatis, 12–15 μ diam.; paraphysibus clavatis, apice 15–15 μ diam.

On *Sphagnum* moss, in storage.

Type locality: Experiment Station, Georgia.

THE NEW YORK BOTANICAL GARDEN

EXPLANATION OF PLATE 14

Center, Photograph of one mature apothecium and several young ones showing habitat; Below, Drawing of one immature apothecium enlarged; Right, Drawing of an ascus when immature; Left, Immature ascus with spores and paraphyses; Above, Three stages in the development of the spore; also trip of ascus showing operculum.

Drawings made with the aid of the camera lucida using a 1 inch eyepiece and a 1/8 objective.

PENICILLIUM GLAUCUM OF BREFELD (CARPENTELES OF LANGERON) REFOUND

C. L. Shear

(WITH 3 TEXT FIGURES)

The fungus described by Brefeld as *Penicillium glaucum* Link with its perithecial form has so far as we are aware not been reported since. In a collection of cultures of soil fungi isolated from soils at Tela, Honduras, by Dr. Otto A. Reinking, 1931, were several species of *Penicillium,* some producing perithecia. One of these was especially interesting (culture No. 2). It was isolated from soil at various depths from the surface to 10 inches, and said to be fairly common. Sub-cultures in tubes of cornmeal agar produced a very thin white superficial growth, and after a day or two an abundance of pale green conidia of *Penicillium.* A few weeks later numerous globose, pale yellowish perithecia appeared. Various sub-cultures were made from ascospores and conidia. These also produced the same *Penicillium* and the same perithecia and ascospores. Cultures were sent to Dr. B. O. Dodge who also verified the connection between the conidia and perithecia, as did Dr. Charles Thom, of the Bureau of Chemistry and Soils. Careful comparison of these cultures with Brefeld's [1] description and plates shows that they agree with his material, so far as it relates to certain of his plates and figures of conidia and perithecia. It is evident, as has been pointed out by others, that some of Brefeld's cultures were mixed, as more than one species of *Penicillium* is described and illustrated. Our cultures, however agree, so far as the conidial stage is concerned, with his plate 8, figure 51, which also shows the conidia arising from the typical germinating ascospores. For purposes of comparison they are reproduced here (FIG. 1). The perithecial form agrees in practically every detail of development with that so fully described by Brefeld, l. c., and

[1] Brefeld, O. Botanische Untersuchunge über Schimmelpilze. II. Die Entwicklungsgeschichte von Penicillium. 1–98, 1874.

illustrated in his plate 6, figures 34–39, with the exception of the statement that the asci are borne in chains (FIG. 2). This is very difficult to demonstrate, but our observations indicate that the asci are borne on very short branches of ascogenous hyphae as described by Dodge[2] for *P. brefeldianum* and form such a dense mass that they have the appearance of being in chains, though Dodge found one or two cases which showed two or three asci in a chain. The development of the perithecium from a sclerotoid

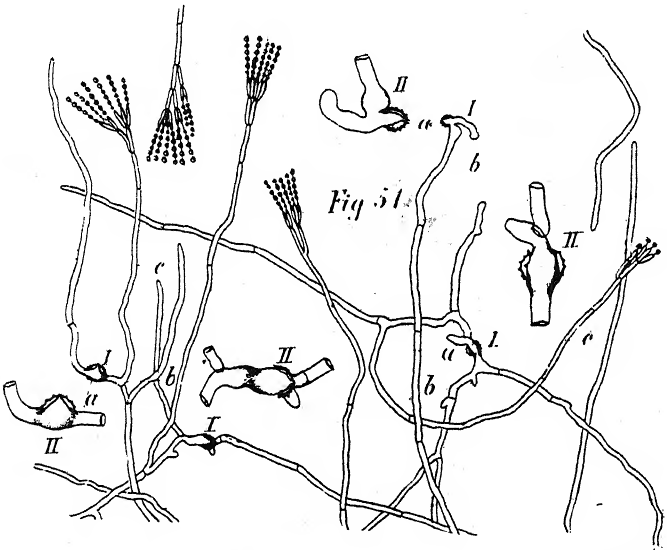


Fig. 1. *Carpenteles asperum.* Copy of Brefeld's Pl. 8, fig. 51, showing germinating ascospores and conidial fructifications.

body as described by Brefeld is very characteristic. The ascospores found in our cultures agree in every particular with those described and illustrated by Brefeld in his plate 7, figures 45 and 46, which are reproduced here (FIG. 3). The spore measurements are given as follows by Brefeld: Conidia 2.5 μ diameter, ascospores $4–6 \times 4–4\frac{1}{2}$ μ. According to our measurements, the conidia are 2 μ in diameter and ascospores $3–4 \times 2–2\frac{1}{2}$ μ. This discrepancy in measurements is, however, easily explained by the

[2] Dodge, B. O. The perithecium and ascus of Penicillium. Mycologia **25**: 90–104, 1933.

discovery of Neuhoff[3] that Brefeld's measurements are always too large, due to an error in the scale he used.

The question now arises as to what name this fungus should bear. Brefeld regarded his fungus as *P. glaucum* of Link. It is now generally agreed that it is impossible to tell with certainty to what particular form of Penicillium Link originally applied this name. It is highly improbable, however, that he had the fungus discussed here.

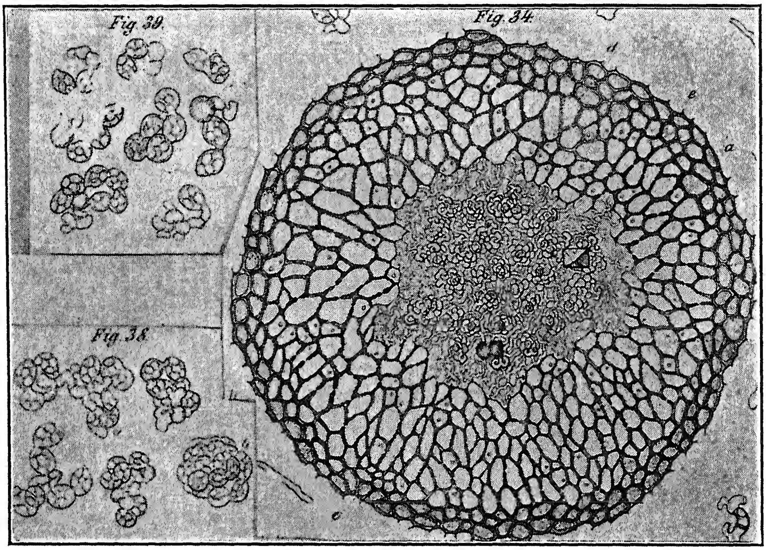


Fig. 2. *Carpenteles asperum.* Copy of Brefeld's Pl. 6, figs. 34–39, showing section of young perithecium and asci and ascospores in different stages of development.

According to the present rules of nomenclature, an ascogenous fungus should bear the generic name applied to its perfect stage. In 1922, Langeron[4] proposed the name *Carpenteles* for species of Penicillium which are known to produce asci and specified as the type of the genus "*P. glaucum* (Link) Brefeld." Langeron, however, did not see the fungus nor verify the connection between

[3] Neuhoff, W. Bot. Archiv. **8**: 253, 1924.

[4] Langeron, Maurice. Utilité de deux nouvelles coupures génériques dans les *Périsporiacés*: *Diplostephanus* N. G. et *Carpenteles* N. G. Comp. Rend. Soc. Biol. Par **87**: 343, 1922.

the conidia and perithecia described by Brefeld and therefore his name has not been adopted by Thom and others. Langeron did not actually publish the combination *Carpenteles glaucum,* but this combination was used by Clements and Shear.[5] In view of the uncertainty regarding the application of *P. glaucum* and the confusion which already exists in its use, it seems best to use another specific name for this ascogenous fungus. We therefore propose the name **Carpenteles asperum** nom. nov., the specific name referring to the spinulose ascospores.

There are several other species of Penicillium which have been shown to have perithecia and spores of the same generic character.

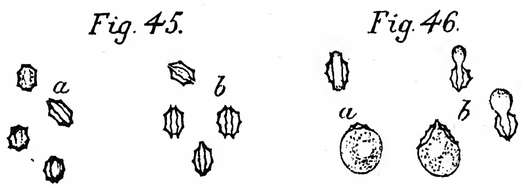


Fig. 3. *Carpenteles asperum.* Copy of Brefeld's Pl. 7, figs. 45 and 46, showing typical ascospores, some of the spores germinating.

Penicillium brefeldianum and its perithecial form described by Dodge, in the paper already cited, is evidently congeneric with *Carpenteles asperum* and should become **Carpenteles brefeldianum** (Dodge) comb. nov. *P. javanicum* van Beijma[6] also belongs here as shown by the studies of Dodge, l. c., which we have verified, and should be **Carpeneteles javanicum** (Van Beijma) comb. nov.

The exact limitations of this genus can not be drawn until the life histories of more of the related species are known. It is clearly related to *Eurotium* which is at present restricted to perithecial forms of *Aspergillus.*

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

[5] Clements, F. E. & Shear, C. L. The genera of fungi, 297, 1931.

[6] Van Beijma Thoe Kingma, F. H. Mykologische Untersuchungen—*Penicillium javanicum* nov. spec. Ver. Kon. Akad. Wet. Amsterdam **26**: 16–19, 1929.

NOTES AND BRIEF ARTICLES

Bartholomew's Handbook

A serviceable compilation of the rusts of North America has been issued recently by Elam Bartholomew, as a "Handbook of the North American Uredinales." In the 238 pages are enumerated all the species in the 7th volume of the N. Amer. Flora, with 12 additional subtropical species. There are 25 new combinations made, and two new names proposed, *Puccinia longipedicellata* and *P. parnassiaecola*. The generic names *Puccinia* and *Uromyces* are restored. Species are arranged alphabetically under each genus with full synonymy, and with page references to the N. Am. Flora, where descriptions are to be looked for. This second edition is an exact reprint of the 1928 edition, to which has been added a complete index of 52 pages. Beside the index this edition adds the two new names already mentioned, two other subtropical species, and in a supplement reference is made to the species of Milesia recently published by Faull, 12 new species in No. 1–2, vol. 31, of Annales Mycologici, and *Uredo Chardoni* Kern. The work is the only complete list of North American rusts and will be found serviceable to collectors and others in a number of ways and especially to those whose herbarium is arranged on the card index plan.

J. C. Arthur.

The Mycological Society of America

The Council of the Society voted at the Atlantic City meeting that the names of the charter members be published in Mycologia. The Constitution states that the charter members are those who joined before or during the formal organization of the Society at Atlantic City. The contract with the New York Botanical Garden, accepted by the Society December 28, 1932, provided that all personal subscribers then receiving Mycologia might become members of the Society if they so desired. The Council ruled that

those expressing the desire to join should be regarded as charter members. The following list, based on these rulings, contains the names of charter members only.

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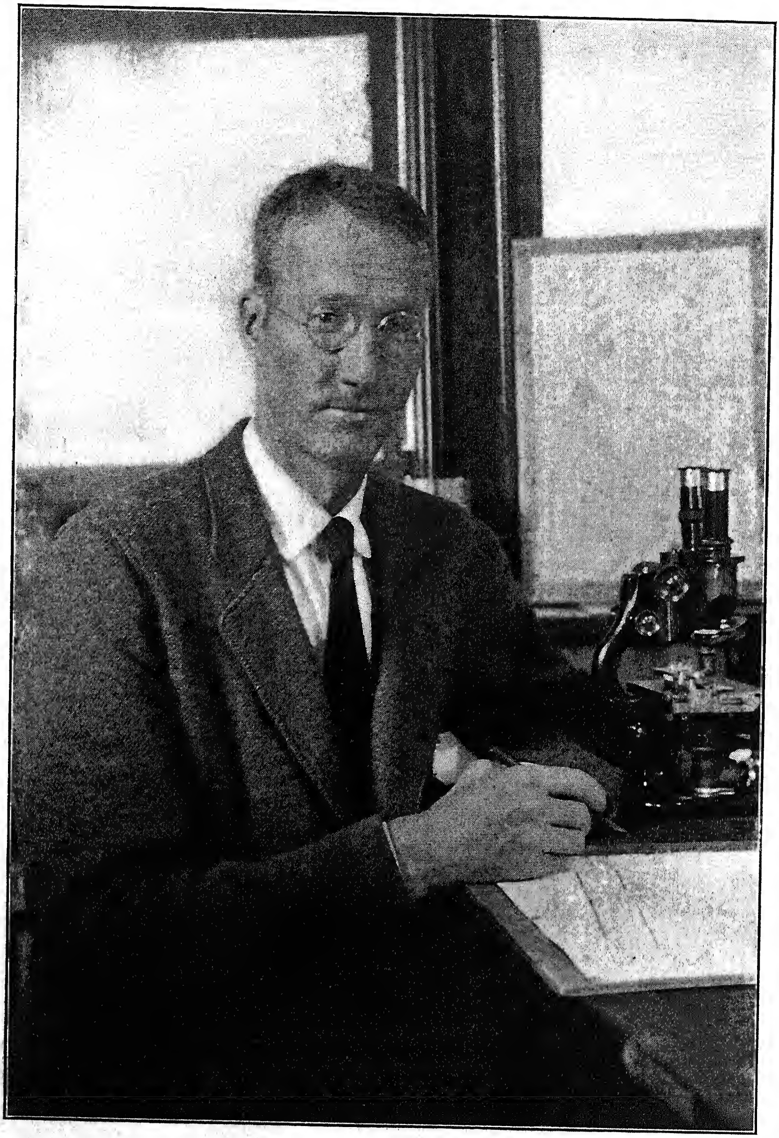

William H. Weston, Jr.

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

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WILLIAM H. WESTON, JR., FIRST PRESIDENT OF THE MYCOLOGICAL SOCIETY OF AMERICA

FREDERICK A. WOLF

(WITH PORTRAIT)

There is little room in the field of science for the operation of the laws of chance. Those events that transpire are rather the resultant of definite causes, admittedly little known or little understood in many cases, but yet they are not merely fortuitous. This is preëminently true in the case of events leading up to and including the birth and establishment of the Mycological Society of America. This organization is in no sense an example of spontaneous generation. Its inception required the presence of a man of the hour. Such a man had to be gifted with the powers of visualizing the benefits that would accrue, in increasing proportions down through the years, to the whole field of mycology if such an organization were in existence, had to be endowed with that accuity and diplomacy so essential for catalyzing and amalgamating sentiment into one concrete unit, and had to be imbued with that dynamic, irresistible, coöperative leadership that carries plans to consummation. This type of personality, this manner of man is the first president of the Mycological Society of America, Dr. William H. Weston, Professor of Cryptogamic Botany, Harvard University. Those who labored with him shoulder to shoulder in this endeavor will comprehend Professor Weston's evaluation of his own efforts in organizing the Mycological Society of America from his characteristic phrase "there wasn't much grief in it."

[MYCOLOGIA for January–February (**26**: 1–112) was issued February 1, 1934]

Properly to appraise the work and influence of a mycological colleague while he yet lives is so difficult as to be almost impossible. A portion only of the qualities for which he may be appraised may be accounted for by the fact that he was trained by such outstanding scholars, teachers, and investigators as Prof. W. G. Farlow and Prof. Roland Thaxter, his predecessors in the same post. Another portion comes from his experiences in mycological and plant pathological investigations that have carried him to the Philippine Islands, Guam, Hawaii, Cuba, and to various parts of the United States. So far as science is concerned these experiences have resulted in a series of studies on the Phycomycetes, primarily on the downy mildews.

In order really to know "Cap," as he is affectionately denominated by those associated with him, one must listen to him in the lecture room as he fluently and enthusiastically presents his materials with a clarity that is intriguing because he never seems to lack for the most acceptable word. From this exactitude in correctness of expression he may suddenly lapse into the use of some current slang so that the student never forgets the point in question. One must also see him daily as he gives unstintingly of his time, experience, and energy in heaping measure to associates and students. If he tires of their numerous intrusions into his office and laboratory to seek counsel, suggestions, references to literature pertinent to their problem, criticisms of experimental procedure, results, or manuscripts, or if he is irritated by them, there are no "external symptoms" of it. His patience, which is monumental, fortified by an unusually keen sense of humor, and his ability to inspire all who come under his influence are an unusual combination of traits that will result in the training and development of a corps of mycologists who will share in the continued enrichment of our knowledge in the field of mycology, and will be an honor to the Mycological Society of America.

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RHOPOGRAPHUS ZEAE ON CORN

R. K. Voorhees

(with plates 15 and 16)

In the spring of 1893, Patouillard [1] reported a species of *Rhopographus* on cornstalks on the ground at Pululahua, Ecuador, which he described under the binomial *Rhopographus Zeae*. Later this description was listed by Saccardo [2] and was emended by Sydow.[3] Aside from these, no other report of this species has been found in the literature. The original description is as follows:

"*Rhopographus Zeae* Pat. nov. sp.—Sur des tiges de mais, a terre. Pululahua. Fevrier. Stromatibus parallelis, linearibus, hysteriiformibus, ½–3 mm. longis, nigris, per rimas parallelas erumpentibus, intus albis; loculis 2–5, vix ostiolatis, connatis, sphaeroideis; ascis clavato-cylindricis, indistincte paraphysatis, 8-sporis (100–150 × 20 μ); sporidiis distichis, fusiformibus, utrinque acutis, melleis, primitus, 1, dein 3, denique 5-septatis, medio constrictis, uno loculo saepe inflatis (33–40 × 6–7 μ)."

In 1928, A. H. Eddins and Erdman West of the Florida Agricultural Experiment Station collected a species of *Rhopographus* on old cornstalks near Gainesville, Florida, which was identified by the latter as *Rhopographus Zeae* Pat. (No. *4770*—Fla. Agr. Exp. Sta. Herb.), but the collection was not reported. During the past three seasons, the writer has found this fungus occurring commonly on old cornstalks in the vicinity of Gainesville.

The *Rhopographus* on corn in Florida agrees with the description of Patouillard, except that thread-like paraphyses are quite distinct (plate 15, fig. 2) and the limits in dimensions of the ascospores of the Florida organism exceed those of his fungus. The ascospores of the Florida fungus measure 30–52 × 6–10 μ, while those of *R. Zeae* as described by Patouillard measure 33–40 × 6–7 μ. In obtaining the dimensions of the spores of the Florida

[1] Patouillard, N. & G. von Lagerheim. Champignons de l'Equateur. Bull. Soc. Myc. **9**: 156–157. 1893.

[2] Saccardo, P. A. Syll. Fung. **11**: 378. 1895.

[3] Sydow, H. Ann. Myc. **13**: 427–428. 1915.

Rhopographus 200 ascospores from material fresh from the field were measured. The parallel, hysteriform stromata are shown in plate 15, figure 1. A typical clavate-cylindrical, 8-spored ascus is shown in plate 15, figure 3. The pointed 1, 3 and 5-septate ascospores, constricted in the middle with one cell frequently swollen are shown in plate 15, figure 4. These spores germinate from both end cells, as shown in plate 15, figures 5, 6, and 7.

No mention was made of a vegetative stage in Patouillard's description of this fungus. However, according to Sydow [4] "the context of the stromata is composed of almost hyaline, delicate, brittle, vertically parallel hyphae 4 μ thick; only the crust above, below, and on the sides is formed of more compact, dark, brownish-olive colored hyphae 12–15 μ thick; on the sides and base these hyphae penetrate between the cells of the matrix." These characters were also observed in the Florida fungus but the hyphae did not exceed 6 μ in diameter.

No report of an imperfect stage of this fungus has been found in the literature, neither has this stage been found on host material in Florida. However, an imperfect stage was produced in culture by planting single ascospores of *R. Zeae* (No. *8057* Fla. Agr. Exp. Sta. Herb.) in petri dishes and test tubes containing corn meal agar. This imperfect stage is typical of the genus *Clasterosporium* Schweinitz. Two species of *Clasterosporium* have been reported on corn. In 1893, Ellis and Everhart [5] named a fungus from corn as *C. olivaceum,* but Saccardo [6] had previously classified another fungus under this binomial. To avoid the confusion of having two different fungi under the same binomial, Saccardo and Sydow [7] renamed Ellis and Everhart's fungus *C. Zeae,* indicative of its host. Saccardo [8] also described another *Clasterosporium* from corn and named it *C. maydicum.*

Since the *Clasterosporium* described in this paper does not correspond with the description of either of the above species, it is considered new and is described as follows:

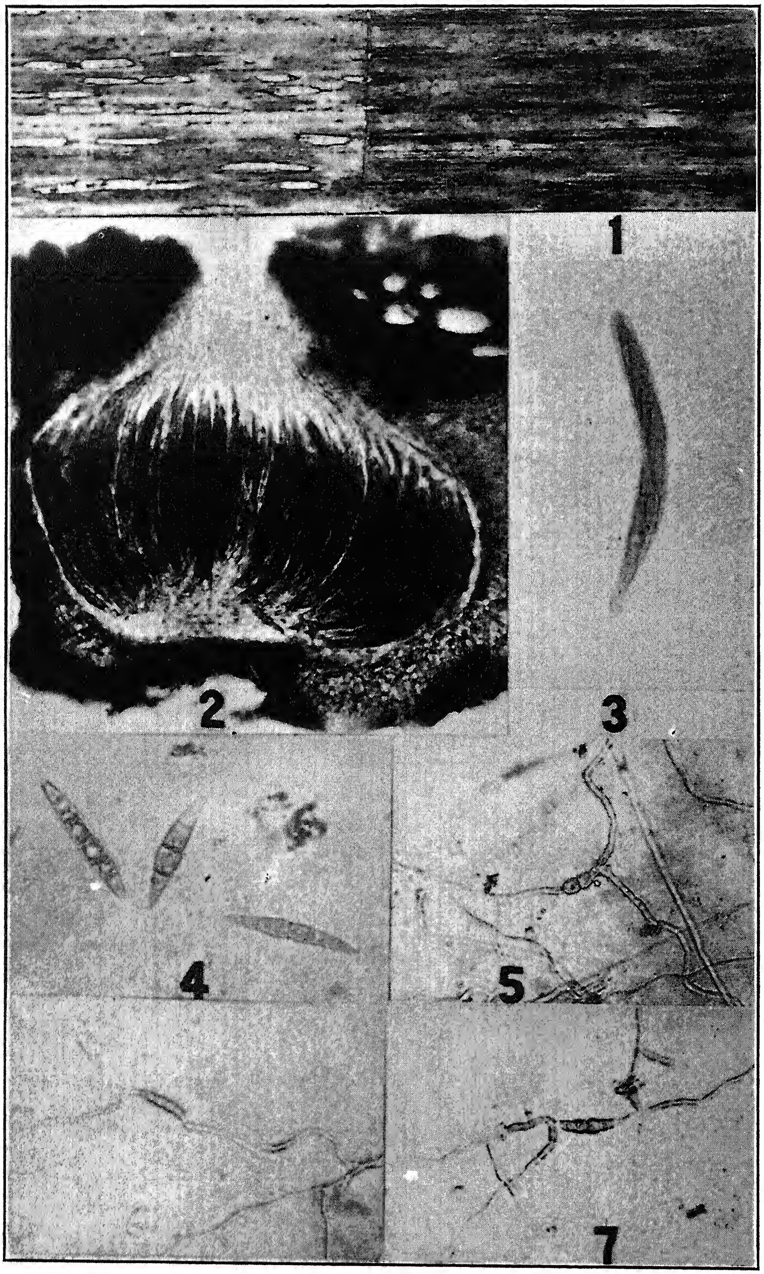
[4] Loc. cit.

[5] Ellis, J. B. & B. M. Everhart. New species of fungi from various localities. Proc. Acad. Nat. Sci. Phila. **45**: 440–466. 1893.

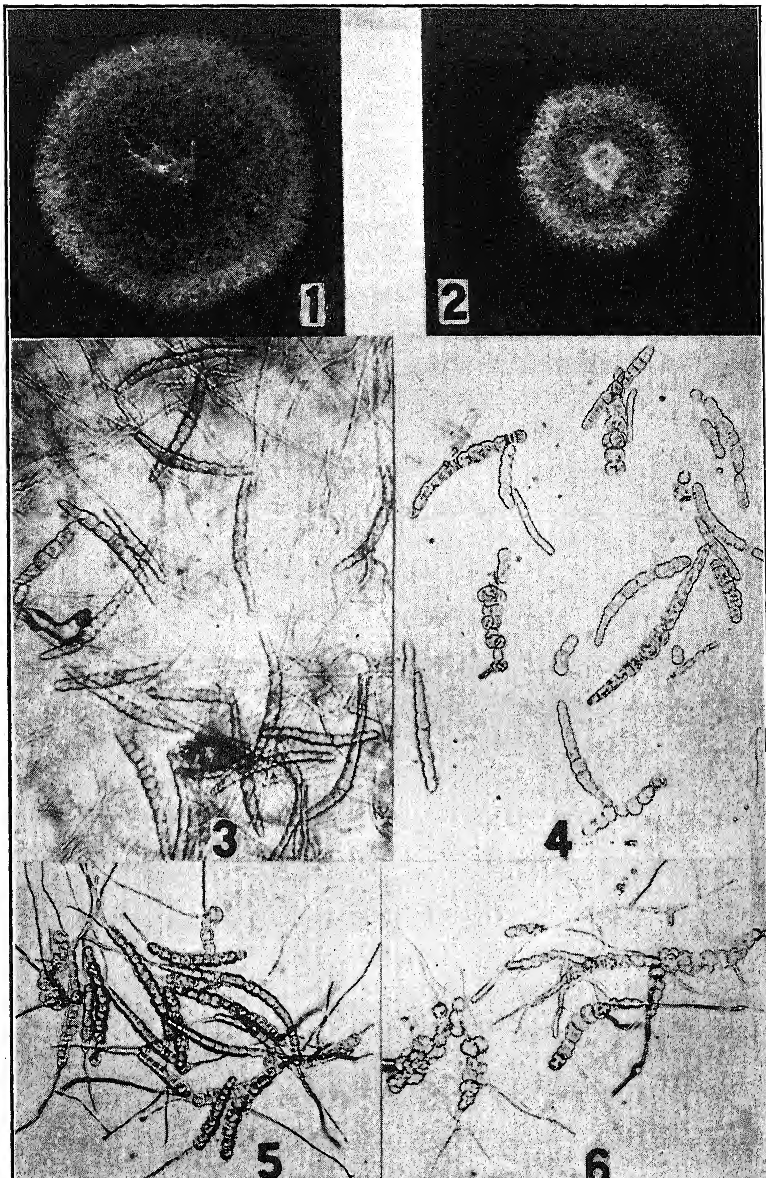
[6] Saccardo, P. A. Syll. Fung. **4**: 382–394. 1886.

[7] Saccardo, P. A. & H. Sydow. Syll. Fung. **14**: 1082–1083. 1899.

[8] Saccardo, P. A. Syll. Fung. **25**: 805–809. 1929.



Rhopographus Zeae



Rhopographus Zeae

Clasterosporium longisporum sp. nov. Mycelium in culture low, medium dense, wooly, almost hyaline, several septate, slightly constricted at septa, frequently anastomosing, 3–5 μ thick; conidiophores short, solitary, slightly darker than hyphae, frequently septate; conidia acrogenous, solitary, elongate, straight or slightly curved, fusoid, muticate, pleuri-septate, constricted at septa, brownish-olive, measuring 70–120 $\times$ 8–20 μ; new cells frequently budding off from parent conidial cells, either of which may germinate (PLATE 16, FIGS. 3, 4, 5, 6).

Hyphae produced from single ascospores or conidia planted on potato-dextrose agar in petri dishes, grow slower than on corn meal agar (PLATE 16, FIGS. 1, 2). Hyphae produced from conidia planted on sterilized corn meal in petri dishes grow slower than on either kind of agar and the growth is higher and more dense.

No infection was produced by this fungus when corn seedlings, stalks and ears were inoculated with either an ascospore or conidial suspension of the fungus growing on corn or wheat kernels. Observations have shown that under natural conditions the fungus does not attack corn stalks until after they have matured. Consequently, no particular damage is caused by it.

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EXPLANATION OF PLATES

PLATE 15

Fig. 1. An enlarged portion of a cornstalk showing the parallel, hysteriform stromata, part of which is cut in half to show the white interior. $\times$ 7.

Fig. 2. A typical perithecium containing asci with thread-like paraphyses. $\times$ 325.

Fig. 3. A typical 8-spored clavate-cylindrical ascus. $\times$ 600.

Fig. 4. Typical pointed 1, 3 and 5-septate ascospores showing constriction in middle with one cell frequently swollen. $\times$ 700.

Figs. 5, 6, 7. One, 3 and 5-septate ascospores respectively, germinating at both ends on potato-dextrose agar. The 3-septate ascospore is shown just before becoming 5-septate (6). $\times$ 250.

PLATE 16

Figs. 1, 2. Single ascospore cultures growing on corn meal and potato-dextrose agar, respectively. $\times$ 2.

Figs. 3, 4. Typical conidia produced from single ascospore cultures growing on corn meal and potato-dextrose agar. Older conidia showing new cells budding off from parent conidial cells (4). $\times$ 250.

Figs. 5, 6. Germination taking place from parent conidial cells and from new cells budded off from parent cells. $\times$ 250.

SPORANGIAL GERMINATION IN THE GENUS MYZOCYTIUM [1]

G. E. Thompson [2]

(WITH 1 TEXT FIGURE)

While searching filaments of *Spirogyra* for members of the lower Phycomycetes, the thallus of a species of *Myzocytium* was found. The method of sporangial germination was later observed and proved to be at variance with those previously described for this genus, since no vesicle is formed at the tip of the exit tube. The zoöspores mature within the sporangium and escape successively in single file.

Thallus. In the young stages the thallus consists of an unbranched mycelial filament which is constricted at regular intervals to form a chain of ellipsoidal cells. At each constriction the narrow channel is plugged with a spherical refractive granule giving the aspect of a thick septum. This is probably a cellulin granule corresponding with those described for the genera *Gonapodya* and *Leptomitus.* Similar refractive granules are scattered throughout the cytoplasm. The individual cells of a mature thallus are oval to ellipsoidal and measure 16–26 × 13–16 μ. The number of cells in the chain varies from five to twelve.

Asexual reproduction. The cells of a chain may all function as sporangia, or as sporangia and male and female gametangia. Each sporangium germinates by a single exit tube, about 3 μ in width, which is slightly constricted where it passes through the host cell wall. At about the time that the exit tube penetrates the wall, the densely granular content of the sporangium rounds up to form small globose bodies showing a slightly oscillating movement. At first this movement is more noticeable towards the center of the mass. In a few minutes it is present in the entire contents of the sporangium. The protoplasm in the exit tube then

[1] Presented at the first meeting of the Mycological Society of America held in Atlantic City, New Jersey, December 28–30, 1932.

[2] The writer wishes to extend his thanks to Professor H. M. Fitzpatrick for many valuable suggestions and for his critical reading of this paper.

begins a definite flow towards its tip, the tip ruptures, and the zoöspores escape successively in single file. As the zoöspore is passing through the orifice of the exit tube its movements are of exceeding interest. It swings rapidly back and forth, each time pulling itself farther from the mouth of the tube. Once free it darts rapidly back and forth in the water. During its attempts to gain freedom a fine strand of protoplasm can be seen trailing out behind.

After most of the zoöspores have escaped, those remaining within the sporangium can be seen darting about until finally they find the way out. Usually all the cells functioning as sporangia germinate simultaneously.

Sexual reproduction. Two contiguous cells function as gametangia in sexual reproduction, one male, the other female. Fertilization is accomplished by the discharge of the contents of the male gametangium through a short terminal tube which penetrates into the female gametangium. No evidence of an oösphere was observed. Following fertilization the content of the female gametangium rounds up and assumes a thin wall to form a resting spore. Later the wall thickens and the globose resting spore, 13–16 μ in diameter, lies free within the female gametangium. It usually contains one or two refractive granules. Following fertilization the two sexual cells are readily distinguishable, the female gametangium being globose to spherical, while the male gametangium is somewhat pyriform.

Discussion. In the genus *Myzocytium* two sorts of sporangial germination have previously been described. In *M. proliferum* Schenk the contents of the sporangium pass out into a thin-walled vesicle in which the zoöspores are delimited. In *M. vermicolum* (Zopf) Fischer, according to Dangeard (**1906**: 210), the zoöspores are delimited within the sporangium and then a group of five or six zoöspores pass out into a vesicle which later breaks, freeing them. The remaining zoöspores then escape singly one after another from the sporangium.

The species described above corresponds most closely in its manner of sporangial germination to *M. vermicolum,* but shows variation in the fact that no vesicle is formed at the tip of the exit tube.

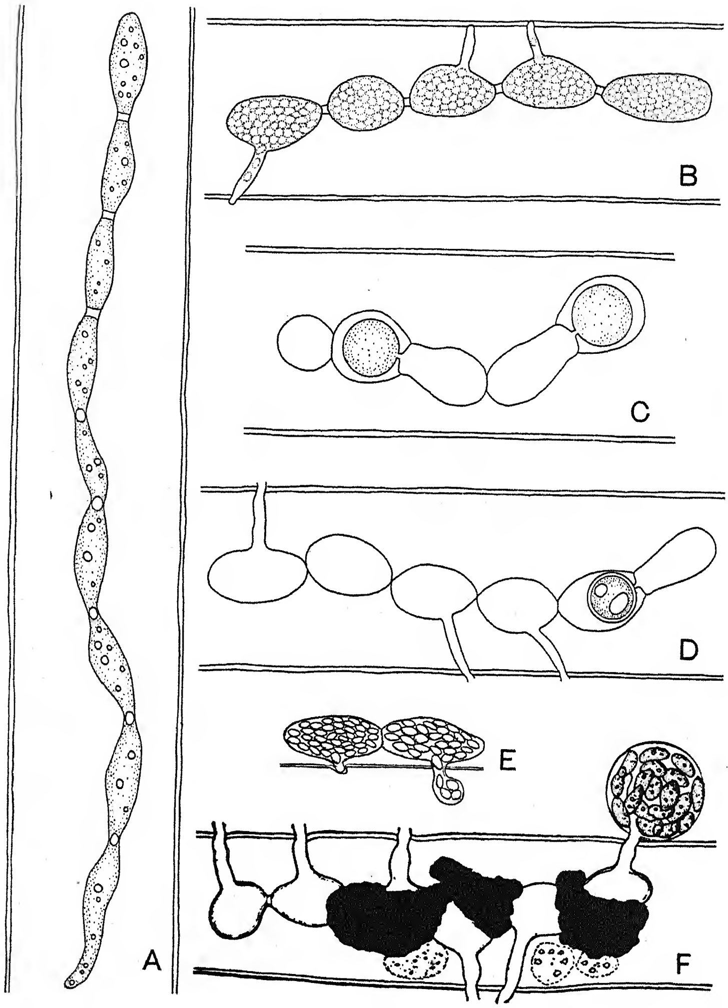

Fig. 1. A, Young thallus showing cellulin plugs at the constrictions. $\times$ 590; B, Chain of mature sporangia with zoöspores ready to escape. $\times$ 590; C, Thallus showing male and female gametangia with immature resting spores. $\times$ 590; D, Chain of empty sporangia with male and female gametangia, the female gametangium containing a mature resting spore. $\times$ 590; E, Sporangial germination of *M. vermicolum* (after Dangeard 1906); F, Sporangial germination of *M. proliferum* (after Zopf 1884).

The writer hesitates to describe this species as new, until he has obtained further information on its morphology and life history. The material was collected in a pond at the Fish Hatchery, Cornell University, during October 1932.

In another species of *Spirogyra,* material of *M. proliferum* was found. In it sporangial germination typical of *M. proliferum* usually occurred. In a few cases, the protoplasmic contents of the vesicle instead of differentiating into zoöspores, rounded up into two or three spherical bodies of varying size, which separated and moved away with a rolling motion. This observation recalls those of Atkinson (**1909**: 335–336) on *Lagenidium americanum.*

Myzocytium proliferum Schenk occurring in various members of the green algae has been reported in literature for America by Martin (**1927**: 188), Graff (**1928**: 168), and Sparrow (**1932**: 288).

Myzocytium vermicolum (Zopf) Fischer occurring in Anguillulidae has not been reported for America. In Europe, Dangeard (**1906**: 202) reports it as being quite common in the bodies of *Anguillules.*

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ANGIOPSORA, A NEW GENUS OF RUSTS ON GRASSES

E. B. MAINS[1]

(WITH PLATES 17–20)

While studying rusts from tropical America, specimens in the Herbarium of the University of Michigan labeled *Uromyces leptodermus* Sydow were examined. Among these a collection was found which was issued as no. *95* in the Reliquiae Holwayanae. The packet contains two leaves of *Lasiacis ruscifolia* (H. B. K.) Hitch. & Chase and is labeled as having only uredinia. Telia are, however, present in abundance (PLATE 17, *A*). They are small crusts which coalesce to form blackish areas 3–15 mm. long and 1–3 mm. wide. A careful search through other collections on *Lasiacis* resulted in finding also in *Uromyces leptodermus* another specimen bearing telia. This was collected on *Lasiacis divaricata* (L.) Hitch. at Utuado, Puerto Rico, 11–8–1915, by F. L. Stevens (*4608*).

Sections show that the telia of these collections are lenticular masses of cells covered by the epidermis. They are arranged in vertical columns up to three to four cells thick in the center of the sorus (PLATE 17, *B*). The cells may be separated from each other only with some difficulty. However, in a mount made by scraping with a scalpel they separate almost as easily vertically as laterally (PLATE 18, *B*). The compact, subepidermal, lenticular telia remind one very much of those of species of *Phakopsora, Bubakia* and *Schroeteriaster*. Species of these genera have not been described on grasses.

However, three species of *Puccinia, P. pallescens* Arth., *P. phakopsoroides* Arth. & Mains and *P. compressa* Arth. & Holw. (not *P. compressa* Diet.) have been described on grasses. These species agree with the rust on *Lasiacis* in many particulars. They all have subepidermal, compact, lenticular telia in which the telio-

[1] Papers of the Botany Department and University Herbarium, University of Michigan No. 435.

spores adhere together laterally. Pedicels are not evident in any of these species.

A comparison of these three species with the collections on *Lasiacis* show that they differ in several important respects. *Puccinia phakopsoroides* and *P. compressa* (PLATE 20, *B*) have well developed paraphyses bordering the uredinia, while a careful study of the rust of *Lasiacis* resulted in finding only a very few hyphoid paraphyses. In this respect the latter resembles *Puccinia pallescens*. *Lasiacis*, however, belongs in the Paniceae of the Poaceae, while Arthur and Fromme (4, p. 278) list species of *Tripsacum* and *Zea* belonging to the Tripsaceae as hosts of *Puccinia pallescens*. One would not expect the same species of rust on such fairly widely separated genera of grasses. The urediniospores of the rust of *Lasiacis* are somewhat larger (15–22 × 22–32 µ) than the measurements given by Arthur and Fromme for *Puccinia pallescens* (13–21 × 20–29 µ). Arthur (2, p. 111) states in his description of *Puccinia pallescens* that the urediniospores of the rust on *Zea Mays* are somewhat larger than those on *Tripsacum*. Through the kindness of Dr. Arthur it has been possible to study a specimen bearing uredinia and telia of this rust collected on *Tripsacum latifolium* Hitch. at Jinotepe, Nicaragua, November 3–7, 1911, by A. S. Hitchcock (*8720*). The urediniospores of this collection are decidedly smaller (12–16 × 18–25 µ) than those of the rust of *Lasiacis*. The urediniospores of the type of *Uredo pallida* Diet. & Holw. which Arthur gives as a synonym are also smaller (14–16 × 16–23 µ). A study of collections of *Puccinia pallescens* on *Zea Mays* received from F. J. Seaver, who collected them in Trinidad in 1921, gives measurements for the urediniospores of 16–20 × 23–30 µ. Since telia have not been found associated with the uredinia on maize it seems doubtful whether this rust on maize should be included in *Puccinia pallescens*. With *Puccinia pallescens* thus interpreted, the rust on *Lasiacis* differs in its larger urediniospores, more luxuriant development of the telia, and the genus of grasses infected. Apparently it is an undescribed species.

Although the three described species have been placed in the genus *Puccinia* their resemblance to species of *Phakopsora* has been noted (5), as is indicated by the name *Puccinia phakop-*

soroides. The addition of another species to this group has made it desirable to restudy the whole situation. Through the kindness of Dr. J. C. Arthur specimens showing the telia of *Puccinia phakopsoroides, P. pallescens* and *P. compressa* have been made available for study. A comparison of the four species shows that they all agree in having subepidermal, compact, lenticular telia in which the spores adhere laterally (PLATES 17, *B;* 19, *A, B;* 20, *A*). No pedicels could be detected. These are the principal characters by which the family Melampsoraceae is usually distinguished from the Pucciniaceae in which the teliospores are free and are borne on a pedicel. Dietel (**9**) has recently placed less emphasis on these distinctions. Although he considers that in general they determine the family he has placed a number of genera in which the teliospores lack pedicels and are more or less laterally adherent in the Pucciniaceae. However, it is generally considered that the genus *Puccinia* contains species in which the teliospores have pedicels and do not adhere laterally. Such species as *Puccinia rubigo-vera* (D. C.) Wint. and *Puccinia Elymi* Westen. have telia in which teliospores are arranged in a compact, more or less lenticular mass long covered by the epidermis. However, when scraped from the leaf and mounted in water, they readily separate from each other. In such species the vertical rows of cells forming the teliospores do not separate easily. In fact they break more easily above or below, rather than at the septa. Although the pedicels in these species are very short, there is no difficulty in detecting them. The species under discussion have telia without evident pedicels and with the spores adhering laterally almost as firmly as vertically. Although the cells of the telium are separable only with some difficulty when a mount is made by scraping with a scalpel, especially if crushed under a cover glass, they tend to separate into single cells (PLATE 18, *B*) indicating that the telia consist of one-celled, catenulate teliospores. They should not, therefore, be placed in the genus *Puccinia.*

As has already been indicated, these species have certain resemblances to species of the genera *Phakopsora, Schroeteriaster* and *Bubakia,* specially in the characters of the telia which have just been described. *Phakopsora* was described by Dietel (**6**, p. 276, **7**, p. 333) based on *Phakopsora punctiformis.* In the de-

scription of this rust, Dietel states that no paraphyses are present and makes no mention of peridia for the uredinia (**6**, p. 276). The telia are described as consisting of one-celled teliospores arranged in a number of layers but united in a lenticular body. Dietel emphasizes the manner in which the teliospores are developed, stating that the spores are not in definite rows but the younger spores are wedged up between the older. Magnus (**10**, p. 130) studied material furnished by Dietel and found both paraphyses and peridia in the uredinia.

Schroeteriaster was described by Magnus (**10**) based on *Schroeteriaster alpinus* (Schröt.) Magnus. Magnus describes the uredinia as without peridia or paraphyses and the telia as consisting of one-celled teliospores without pedicels. These are united together in a many-layered lenticular crust.

Bubakia was proposed by Arthur (**1**, p. 338) for those rusts with uredinia without paraphyses or peridia and with teliospores compacted into a subepidermal telium more than one layer thick. *Bubakia Crotonis* (Burr.) Arth. was selected as the type species.

A comparison of the four grass rusts with species of these three genera has resulted in the discovery of several important differences. Sections of uredinia of *Schroeteriaster alpinus* show that these have a broad, flat spore-bearing surface which is early exposed and only bordered by a slight fringe of ruptured epidermis. The urediniospores are borne on well developed pedicels. The uredinia of the grass rusts under discussion are much smaller, more or less covered by the epidermis which is irregularly slit and the pedicels are either very inconspicuous or absent. The telia of *Schroeteriaster alpinus* consist of one-celled teliospores fairly firmly compacted into lenticular crusts. A careful study of the telia has resulted in finding that the teliospores are not catenulate but are produced singly on pedicels. The pedicels are hyaline and very delicate and apparently are for the most part soon destroyed by the pressure of the crowded teliospores. They can be detected for the younger teliospores, specially when such develop near the margin of uredinia where they are not subjected to pressure. Occasionally pedicels can be traced downward from the teliospores in the upper part of the telium. The younger telio-

spores develop between the older and are pushed up between them and form a compact mass and separate with some difficulty.

Magnus (**10**, p. 131) considered that *Schroeteriaster* belonged in the Pucciniaceae close to *Uromyces*. The Sydows (**11**, p. 399), Arthur (**1**, p. 338), and Dietel (**8**, p. 548) have placed it in the Melampsoraceae close to *Phakopsora*. Dietel later (**6**, p. 84) considers it in the Pucciniaceae next to *Uromyces*. The presence of pedicels in the telia supports the latter arrangement. The genus is apparently monotypic and differs from *Uromyces* only in the compact lenticular telia with more or less adherent teliospores.

In many respects the uredinia of the grass rusts resemble those of *Phakopsora* and *Bubakia*. Unfortunately it has not been possible to study material of *Phakopsora punctiformis* Diet. but a comparison with *Bubakia Crotonis* (Burr.) Arth. and several species which have been placed in *Phakopsora* shows agreement in the presence of the overarching epidermis and the apparently sessile urediniospores.

However, the telia of the grass rusts show an important difference from those of these genera. Dietel in his description of *Phakopsora* emphasizes the fact that the one-celled teliospores are not catenulate but that the younger develop between the older and wedge in between them. A study of the type of *Bubakia* has resulted in finding a similar development for its teliospores. The younger spores apparently force the older upward in their development and by their adherence they form lenticular crusts. The grass rusts with their catenulate teliospores thus form a group which differs sufficiently to be considered a separate genus. For this the name *Angiopsora* is therefore proposed.

Angiopsora gen. nov.

Uredinia minuta, subepidermalia, diu tecta, praedita paraphysibus aut nullis; urediniosporae echinulatae, solitariae, sine conspicuis pedicellis. Telia subepidermalia; teliosporae coloratae, leves, unicellulares, catenulatae, 2–4 superpositae, stratum compactum lentiforme formantes, catenulis lateraliter arcte coalitis, sine pedicellis.

Species typica: *Angiopsora lenticularis.*

The rust on *Lasiacis* is an undescribed species and for it the name *Angiopsora lenticularis* is proposed.

Angiopsora lenticularis sp. nov.

II.Urediniis amphigenis, minutis 0.2–0.4 mm. subepidermalibus, diu tectis; urediniosporis sessilibus, ellipsoideis vel obovoideis, $15–22 \times 22–32\ \mu$, membrana tenui praeditus, $1–1.5\ \mu$, echinulatis, poris inconspicuis.

III. Teliis hypophyllis, minutis, 0.2–0.3 mm., atro-brunneis, aggregatis in macula 3–15 mm. longa, 1–3 mm. lata; teliosporis variabilibus, angulatim ellipsoideis vel oblongis, $11–16 \times 16–32\ \mu$ crassis, ad apicem leniter incrassitis, $2–4\ \mu$, flavo-brunneis, sine pedicellis.

Lasiacis ruscifolia (H. B. K.) Hitch. & Chase, Guayaquil, Ecuador, July 31, 1920, E. W. D. and Mary M. Holway, (*801*) Reliq. Holw. *95*. II III, type.

Lasiacis divaricata (L.) Hitch. Utuado, Puerto Rico, Nov. 8, 1915, F. L. Stevens, (*4608*) II III.

The collection on *Lasiacis ruscifolia* has abundant telia which in many cases coalesce to form conspicuous blackish areas (PLATE 17, *A*). The telia on *Lasiacis divaricata* are few and scattered probably indicating that the formation of telia had just started when the collection was made. Otherwise the two collections are very similar. As indicated by plate 17, *B* the teliospores show no evidence of pedicels. The catenulate arrangement of the teliospores is responsible for a certain resemblance to those of *Puccinia*. However, as has already been discussed, the separation of cells of the telium when crushed indicates that each cell should be considered a teliospore (PLATE 18, *B*).

The uredinia are more or less bullate (PLATE 18, *A*) and the overarching epidermis is ruptured as a more or less irregular slit. A careful study indicates that thin-walled, hyphoid paraphyses are occasionally formed but these are so few and difficult to find that they are of little value as a diagnostic character. In sections of the uredinia, some evidence was found of a fungal tissue immediately beneath the overarching epidermis. From dried herbarium specimens it is difficult to determine its nature since it apparently is soon compressed by the pressure of the developing urediniospores. It appears to be a thin layer of hyphae and probably is responsible to some extent for the persistence of the overarching epidermis. It possibly may be the remnant of a delicate thin-walled peridium.

The three species belonging to this group of rusts which have been assigned to *Puccinia* should be transferred to the genus *Angiopsora* with the following modifications.

Angiopsora pallescens (Arth.) comb. nov.

Uredo pallida Diet. & Holw.; Holw. Bot. Gaz. **24**: 37. 1897.
Puccinia pallescens Arth. Bull. Torrey Club **46**: 111. 1919.
Dicaeoma pallescens Arth. & Fromme, N. Am. Flora **7**: 276. 1920.

As already stated, Arthur and Fromme have included a tropical *Uredo* on maize in this species. This has larger urediniospores than the rust on *Tripsacum* and should be excluded until telia are found by which it may be properly placed. As here interpreted the urediniospores are 12–16 × 16–25 μ. As in the previous species, no pedicels are evident. The spores, however, are apparently borne singly. By careful search a few hyphoid paraphyses can also be found. A thin hyphal layer similar to that noted in the previous species can be demonstrated beneath the overarching epidermis. The teliospores are like those in *A. lenticularis*. They measure 10–16 × 10–26 μ and form chains 18–70 μ long which adhere laterally to form compact sori. Otherwise the species is as described by Arthur and Mains. (1919). This rust has been reported on *Tripsacum latifolium* Hitch. and *T. lanceolatum* Rupr. from Mexico, Guatemala, Nicaragua, and Salvador.

Angiopsora phakopsoroides (Arth. & Mains) comb. nov.

Puccinia phakopsoroides Arth. & Mains, Bull. Torrey Club. **46**: 412. 1919.
Dicaeoma phakopsoroides Arth. & Fromme, N. Am. Flora **7**: 295. 1920.

As previously described (**5**), the teliospores were considered to be multicellular. Instead of this they appear to be unicellular, cuboid, 8–14 × 10–16 μ, catenulate in chains 20–40 μ long. The appearance of a colorless layer, described as continuous around the 2- to 3-spored chains, is probably due to the compression and adherence produced by the pressure of the compact telium. No

pedicels have been distinguished. The uredinia are bordered by abundant, peripheral, incurved paraphyses. They are also covered over, except for an irregular slit, by the overarching epidermis. The urediniospores are apparently borne singly. No pedicels could be distinguished in the herbarium specimens studied. This rust has been reported on *Olyra latifolia* L. from Cuba, Puerto Rico, and Ecuador.

Angiopsora compressa (Arth. & Holw.) comb. nov.

Puccinia compressa Arth. & Holw.; Arth. Proc. Am. Phil. Soc. 64: 157. 1925. (Not *Puccinia compressa* Diet. Ann. Myc. 5: 245. 1907.)

Arthur in his description of this species likens it to *Puccinia phakopsoroides* and similarly describes the vertical rows of spores as two-celled teliospores, noting however that each cell was "rounded off at the ends as if it were an independent spore." He also comments on the lack of pedicels and the lateral adherence of the rows. As in the previous species the teliospores are here interpreted as one-celled, 12–14 $\times$ 20–26 μ, in rows of 1–3, usually 2, 40–50 μ long. The uredinia are bordered by incurved paraphyses as described by Arthur. Apparently there is also a thin hyphal layer under the over-arching epidermis as in *A. lenticularis*. This rust was described on *Paspalum elongatum* Griseb. from Bolivia.

Without more information in regard to the development of these species it is difficult to place this genus in its proper relationship. The telia have both of the main distinctive characters which have been used to separate the Melampsoraceae from the Pucciniaceae, *i.e.* lack of pedicels and lateral adherence of the teliospores into compact crusts. However, in the Melampsoraceae very little information is available concerning the development in those species having catenulate teliospores. In genera with long chains such as *Cronartium* apparently the chains elongate by the successive divisions of the basal cell. Probably this also occurs in the genus *Cerotelium* which with its shorter chains of spores more nearly resembles *Angiopsora*. It seems somewhat doubtful whether *Cerotelium* with its colorless thin-walled teliospores in

chains, which are somewhat loosely held together laterally, is directly related to *Angiopsora* with its dark colored teliospores and compact sori.

The very short chains of teliospores of *Angiopsora* suggest that the development may have taken place by the successive divisions of the uppermost cell, the cell cut off from the basal cell dividing and the upper cell of the two produced again dividing to form the chain. It is possible that the basal cell sends out side buds which repeat the same process, although the rather uniform palisade arrangement of the spore chains indicates that this is doubtful. This type of development is very similar to that which takes place in the formation of teliospores of *Puccinia* except that in *Angiopsora* the resulting cells are more loosely held together in the chain, and are more firmly adherent laterally and are apparently without a pedicel. In *Angiopsora* all the cells produced apparently develop into spores. This, however, cannot be determined with certainty without a careful study of fresh material. If the supposition suggested here is correct *Angiopsora* would show a distinct tendency in the development of its telia toward *Puccinia*. If it should happen that the basal cell of the spore-chain should remain undeveloped and inconspicuous, in other words forming a poorly differentiated pedicel-cell, the genus would be placed much nearer to *Puccinia*. The fact that it is the only genus besides *Puccinia* and *Uromyces* occurring on grasses would lend some weight to this hypothesis.

This, however, would not necessarily interfere with a close relationship with *Bubakia* and probably *Phakopsora*. They would stand in a relationship to *Angiopsora* similar to that between *Uromyces* and *Puccinia*. In *Bubakia* and *Phakopsora* the cell cut off from the basal cell does not continue to divide, and the developing younger spores, possibly from lateral buds, force the older upward, and the adherence of the spores results in a somewhat similar appearing lenticular telium.

Schroeteriaster might be considered a link between *Phakopsora* and *Bubakia* on the one hand and *Uromyces* on the other. However, there are several objections to this, the principal one being the flat, open uredinium with well developed pedicels for the urediniospores. The differentiated, although fragile, pedicels of

the teliospores place it much closer to *Uromyces* than to *Bubakia.* Rather it would appear that *Schroeteriaster* is an offshoot from *Uromyces* in which a pronounced lateral adherence of the teliospores has developed resulting in a crust.

While *Angiopsora* shows a certain tendency in the direction of *Puccinia,* the uredinia would indicate that the relationship is probably not as close as that to *Bubakia.* The bullate, long-covered uredinia with bordering paraphyses, and possibly peridia, and sessile urediniospores give additional evidence of very close relationship to *Bubakia* and *Phakopsora.* It is possible that inconspicuous pedicellate cells may be formed. But this also is a situation which more commonly occurs in the Melampsoraceae than in the Pucciniaceae. It therefore seems best from the evidence available at present to place the genus *Angiopsora* in association with *Bubakia* and *Phakopsora* in the Melampsoraceae, recognizing that it shows a certain tendency of development toward *Puccinia* in the Pucciniaceae.

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EXPLANATION OF PLATES

PLATE 17

A, Two leaves of *Lasiacis ruscifolia*, Reliq. Holw. 95 (type specimen), showing telia of *Angiopsora lenticularis*, aggregated in blackish groups; *B*, Section through a telium of *Angiopsora lenticularis* showing catenulate arrangement of teliospores in the subepidermal crusts.

PLATE 18

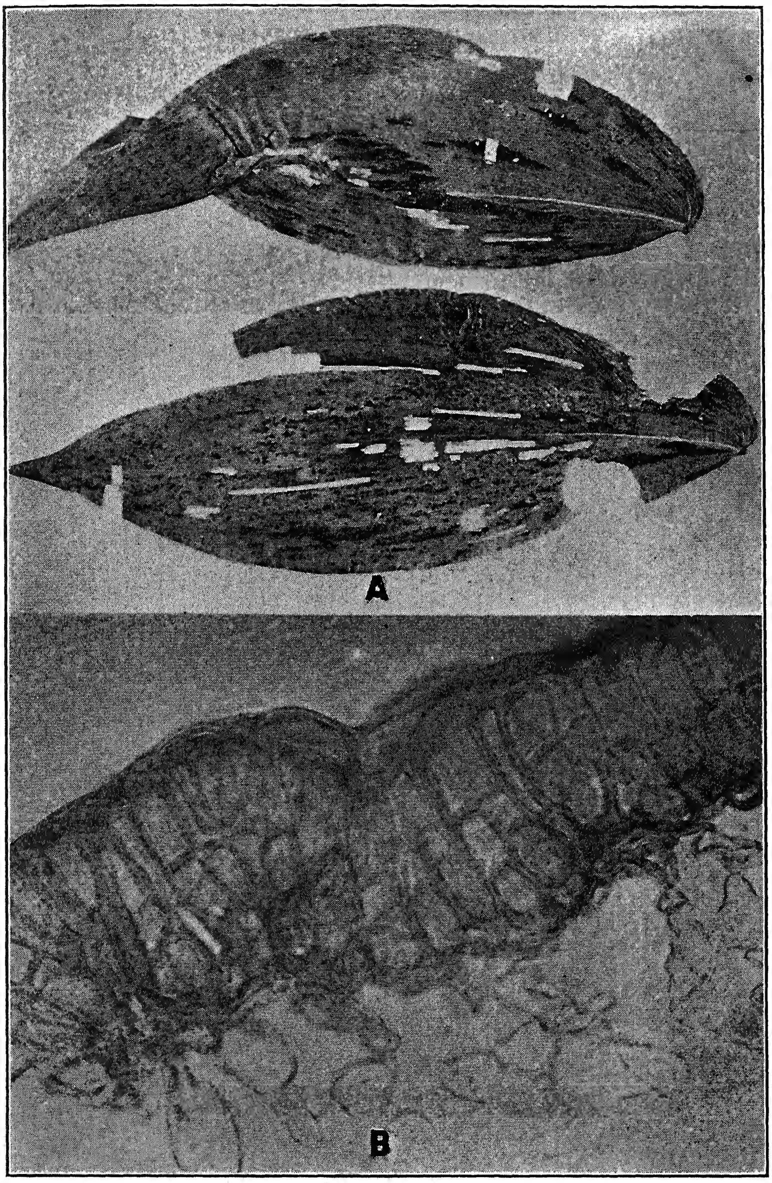
A, Section through a uredinium of *Angiopsora lenticularis* showing the overarching epidermis. There is some evidence of a thin hyphoid layer beneath the epidermis; *B*, A telium of *Angiopsora lenticularis* crushed out under a coverglass, showing the separation into one-celled teliospores.

PLATE 19

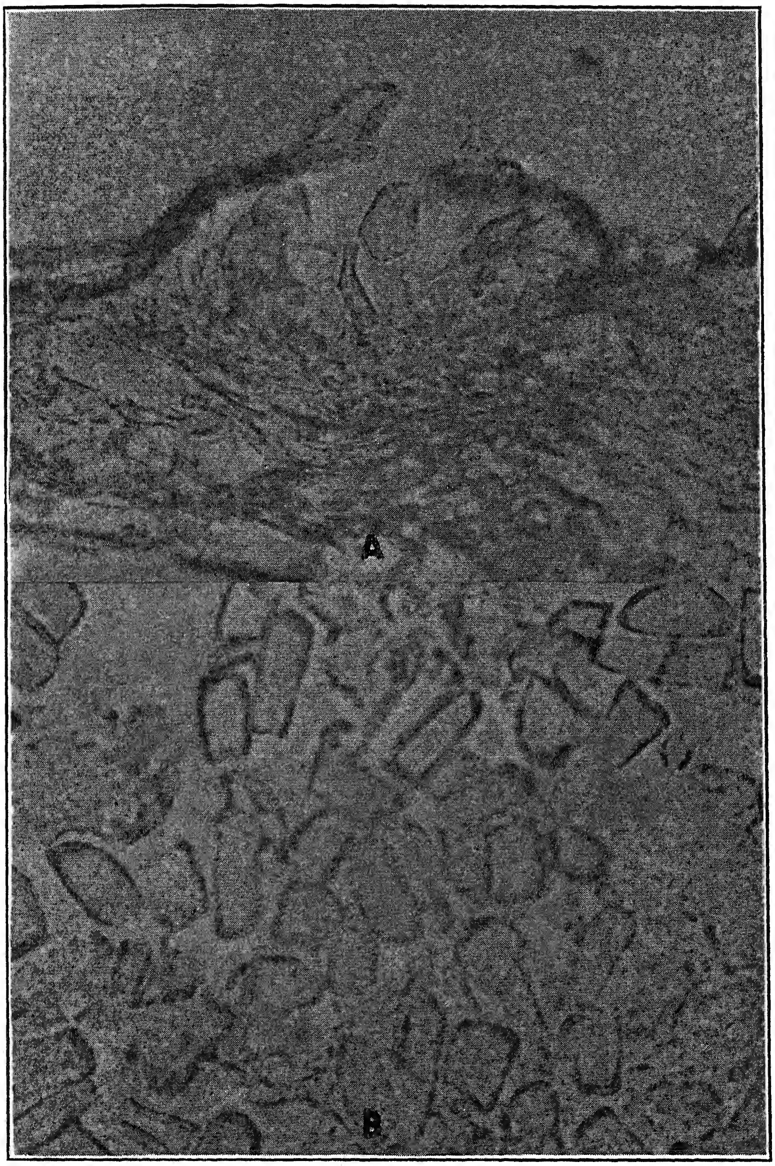
A, Section through a telium of *Angiopsora phakopsoroides* showing the arrangement of the catenulate teliospores in a subepidermal, compact layer; *B*, Section through a telium of *Angiopsora pallescens* showing a similar arrangement.

PLATE 20

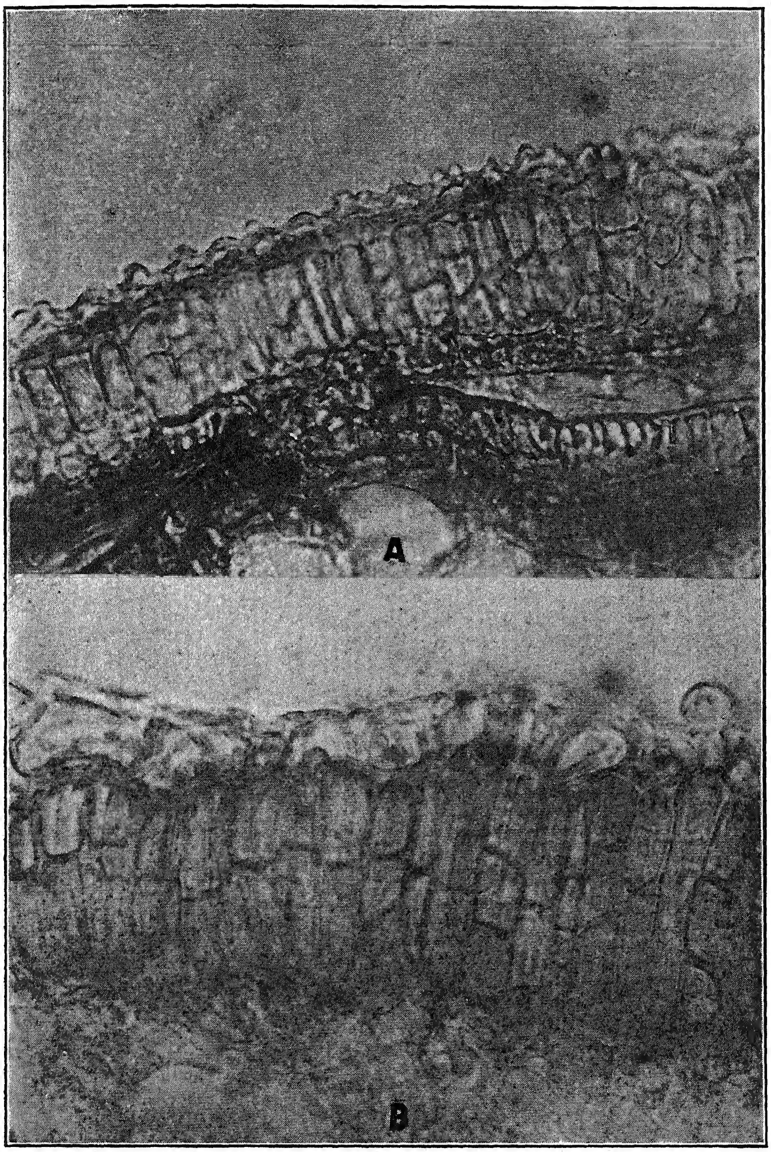
A, Section through a telium of *Angiopsora compressa* showing the compact arrangement of the two-spored chains. Part of the epidermis was torn away in sectioning; *B*, Section through a uredinium of *Angiopsora compressa* showing the colorless, incurved paraphyses at the margin of the sorus.



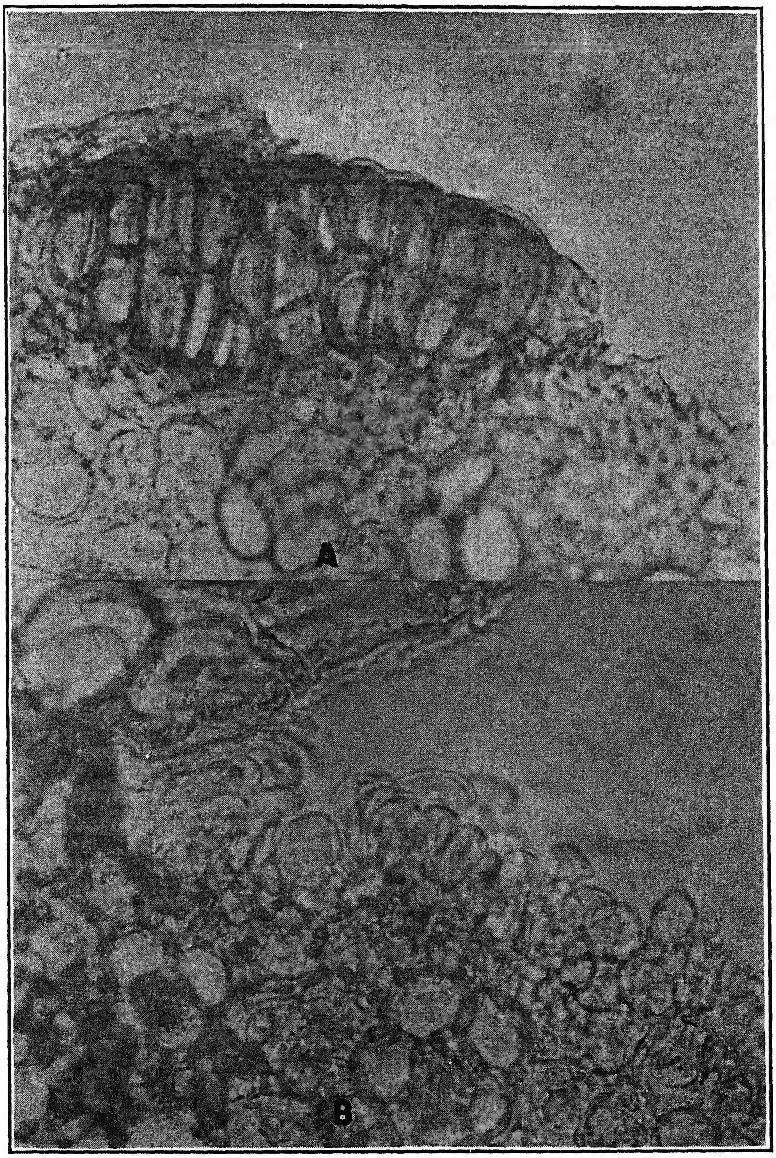
ANGIOPSORA LENTICULARIS



Angiopsora lenticularis



ANGIOPSORA PHAKOPSOROIDES
ANGIOPSORA PALLESCENS



ANGIOPSORA COMPRESSA

MYCOTYPHA MICROSPORA ISOLATED FROM CHAENOMELES LEGENARIA

W. F. Cherry

Aline Fenner Kempton described and illustrated a new genus of Mucoraceae (*Mycotypha microspora*) in Mycologia **24**: 187–198. 1932. She found this organism growing in mixed cultures from the isolation of a pathogen of an orange.

In an effort to isolate a fungus causing a spotting of the fruits of *Chaenomeles legenaria* (Japanese Flowering Quince) the writer discovered *Mycotypha microspora* growing in mixed cultures from the attempted isolation. The fruits from which the isolation was obtained were grown near Lafayette, Indiana. Potato-dextrose agar was used as a culture medium from which all measurements and description of the fungus were made.

The vegetative hyphae of the fungus are much branched containing a dense granular protoplasm and many vacuoles. There is a lack of uniformity in the diameter of the mycelium especially near the attachment of the secondary branches.

The cat-tail like fructifications at first are white with a pinkish tinge which becomes darker as they mature, gradually changing to a dusky slate gray. Old cultures are dark brown in color. The fructifications grow compactly together and stand erect.

The capitella vary in length from 30 to 450 microns and from 16 to 28 microns in width with spores removed. The conidia drop off at maturity leaving the hollow cylindrical head exposed. The naked capitellum appears marked with many orifices but close examination shows numerous scars where the spores were attached. The conidia vary from ovoid to spherical in shape and range from hyaline to bluish green in color. In size they vary from 4 to 6 microns. Germination was noted to take place 4 to 5 hours after being sown on the potato-dextrose agar at room temperature.

Correspondence and exchange of cultures between the writer and Aline Fenner Kempton demonstrated conclusively that the

fungus isolated from the fruits of the Japanese flowering quince was identical with the one isolated from oranges.

The author wishes to acknowledge the helpful suggestions of Dr. C. L. Porter of the Biology Department of Purdue University under whose direction the work was performed.

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ORGANS OF CAPTURE IN SOME FUNGI PREYING ON NEMATODES

CHARLES DRECHSLER

In a recent paper in this journal Sherbakoff (14) gave an interesting account of a fungus capturing nematodes by insnaring them in ring-shaped structures, later growing into the animals and ultimately consuming them. The ring-shaped structures he interpreted as conidia, and in the absence of any genus known to produce annular spores, he erected a new genus *Anulosporium*, for which he claimed a place near *Helicomyces* Link and *Helicoon* Morgan among the mucedinous Helicosporae. As the merit of the new genus is contingent on the essential character of the annular bodies, whether they really represent or do not represent conidia, it may not be inappropriate to direct attention more particularly to two nema-capturing Hyphomycetes, the brief characterizations and rather meager synoptic illustrations of which might readily be overlooked in the several summaries (4, 6, 7) dealing with nearly a score of predacious fungi.

The two Hyphomycetes in question produce annular structures which as the figures of them (4, *fig. 6, B*; 7, *fig. 16, B*) indicate are very closely similar in dimensions and in manner of attachment. If the comparison is extended to Sherbakoff's description and photomicrographs (14, *pl. 35, A–C, E–G, I–K, N*) of the bodies he interpreted as the conidia of his *Anulosporium nematogenum*, a similarly striking correspondence is evident. In both species the annular structures function in insnaring the nematodes without active or serious constriction, and while rather inconspicuous differences are observable in the initial development within the animals immediately following perforation of the integument, similarity is again evident both in the frequently delayed penetration, and in the somewhat lingering decline of the prey. A doomed animal, often retaining its vigor for some hours after capture, may tear the encircling loop from its slender attachment, frequently to be caught in a second loop, and sometimes following renewed

struggles and a second liberation, even in a third. Sherbakoff's photomicrographs (**14**, *pl. 35, B, J, L 3*) showing two or three annular structures encircling captured animals, testify to a parallelism in predacious habit in the fungus reported by him.

The fact that is of most direct interest here is that the two fungi under consideration produce under favorable conditions conidiophores and conidia of types long recognized. In one of the fungi the conidia are spindle-shaped with rounded ends, measure on an average approximately 47 μ in length and 8.5 μ in diameter, contain mostly 4 and less frequently 5 septa, and are borne in loose capitate arrangement in numbers usually between 3 and 7, terminally on erect conidiophores measuring mostly about 0.3 mm. in height, 3.0 μ in diameter at the base and 1.5 μ in diameter near the tip (**4**, *fig. 6, A*). The principal details of morphology are therefore in tolerable agreement with those attributed to *Dactylaria candida* (Nees) Sacc. (= *Dactylium candidum* Nees) in the general works of Saccardo (**13**, p. 195) and of Lindau (**10**, p. 416), in both of which cognizance is taken of Bonorden's (**3**, *fig. 139*) illustrations in regard to spore septation and also of Oudemans' (**12**) measurements of spore dimensions. Some misgivings concerning the possible identity of the American with the European fungus are aroused in considering the manner of attachment of the conidia, since in my fungus the loose capitate arrangement is brought about by the spores being borne on short terminal spurs, whereas the various figures of *D. candida* show no indication of such modifications at the tip of the sporophore. Yet as the figures of *D. candida* are on a scale of magnification much too small to record such modifications, if any had been present in the material used, without considerable exaggeration, anything like a rigorous interpretation of these illustrations would seem ill-advised. It is hardly necessary to mention, moreover, that the cavalier draughtmanship prevalent in the earlier days of mycology was primarily concerned with the grosser aspects rather than with the more intimate details of external form.

The occurrence of *Dactylaria candida* on the inner surface of bark separated from an old oak stump, as reported by Bonorden (**3**, p. 82), is not at variance with a presumption that it is often or even habitually predacious on nematodes in nature, as I have found

pieces of decaying bark that have been in contact with moist soil a prolific source of many nema-capturing fungi, among the most frequent of these being, indeed, the very one considered probably identical with Nees von Esenbeck's species. Oudemans' discovery on goat dung of the fungus to which he attached the same binomial, provides a circumstance perhaps even more suggestive of a predacious habit, especially as he reported in the same paper, and it may be assumed as a result of similar handling of gross cultures, the occurrence of *Arthrobotrys oligospora* Fres. also on goat dung, and the discovery of his *Monacrosporium elegans* on rabbit dung. The conidium of the latter fungus (12, *fig. 9*) shows such an obvious family resemblance to that of one of the species of *Dactylaria* figured earlier (4, *fig. 5, A*) as well as to the spores of two of the species of *Monacrosporium* (6, *fig. 12, A;* 7, *fig. 17, A*), that the presumption of a similar biological relationship is difficult to avoid.

From a consideration of morphological similarities and of the character of the substratum in encouraging the multiplication of nematodes, a fair presumption can be entertained that *Monacrosporium subtile,* another fungus described by Oudemans from rabbit dung in the same paper with the species already cited, represents likewise a nema-capturing form. One (12, *fig. 10 a*) of the two conidia figured by Oudemans shows at least a moderate resemblance to the conidia produced by the second of the two fungi isolated by me that capture nematodes in delicate, solitary, non-constricting loops (7, *fig. 16, B, C*), though because of the pronounced clavate shape of the other spore (12, *fig. 10 b*) figured by the Dutch investigator, there would appear to be somewhat greater likelihood of the plants being congeneric rather than conspecific. For the conidia of the American fungus in question are narrowly fusoid, suggesting in shape and septation the macroconidia of various species of *Fusarium,* yet lacking the curious basal modification usual in the latter, and being formed only on discrete conidiophores (7, *fig. 16, A*).

As has been mentioned the non-constricting loops or rings present in the fungus provisionally identified as *Dactylaria candida* are so closely similar to those produced by the *Fusarium*-like species of *Monacrosporium* just discussed, that the two plants could not well be distinguished by these structures. Apart from

the very obvious differences in conidiophores and conidia, the former may be recognized by the production individually on delicate stalks, within the substratum, of characteristic globose cells, about 4 or 5 μ in diameter (4, *fig. 6, B*), the equivalent of which I have not observed so far on the mycelium of the latter in nematode-infested plate cultures. These cells manifestly correspond to the "globular bodies" described by Sherbakoff for his *Anulosporium nematogenum,* and that with such exactness that specific identity is very strongly suggested. Even if, as seems probable, similar solitary non-constricting loops and similar globose bodies will ultimately be found associated in other nema-capturing fungi—and undoubtedly more than a few of the supposed saprophytes described from excrement of various animals or from decaying plant remains, that are compiled in the "Sylloge fungorum" in such genera as *Arthrobotrys* Corda, *Trichothecium* Link, *Cephalothecium* Corda, *Dactylaria* Sacc., *Dactylella* Grove, *Dactylium* Nees and *Monacrosporium* Oud. will prove to be predacious—it seems doubtful whether a more thoroughgoing agreement will ever be brought to light.

The globose cells which Sherbakoff sets forth as representing an early stage in the development of the annular loops, can, I believe, be more appropriately interpreted as constituting in themselves completed organs of capture, independent of the loops, and designed to take smaller, or in any case, less vigorous prey. In flourishing agar plate cultures of the fungus tentatively identified as *Dactylaria candida,* annular organs can be seen in all stages of development including the earliest stages, but the resemblance of such earliest stages to the globose bodies is certainly not impressive. Again, in the *Fusarium*-like species of *Monacrosporium* similar loops are formed, though globose cells have not been seen associated with them.

The true character of such globose cells would seem revealed in the larger and more robust but otherwise apparently similar structures (4, *fig. 7, B*) occurring in a fungus, the solitary conidia of which, typically broadly spindle-shaped and 4-septate, and measuring 30 to 65 μ in length by 13 to 18 μ in diameter, are borne terminally on erect conidiophores approximately 0.2 mm. in height (4, *fig. 7, A*). As this fungus was derived frequently from pieces

of rotten wood, a rather satisfactory agreement with *Dactylella ellipsospora* described by Grove (**9**) from England in 1886, prevails with respect to source as well as to morphology of conidiophore and conidium.[1] In any case the globose bodies here are functional in the capture of nematodes in causing them to adhere by means of an adhesive substance, which then soon becomes visible as a cushion-like deposit of transparent, colorless, gelatinous material through the middle of which a narrow process is thrust forth to perforate the animal's integument (**4**, *fig. 7, C*). They correspond well in shape, dimensions and performance, to the subspherical structures described by Zopf (**16**) for his *Monosporidium repens,* which he found abundantly destructive to nematodes in rabbit dung. Either because this nema-capturing fungus of Zopf's failed to produce conidia, or probably because the opaque substratum obscured the organic connection between the conidiophores and the globose bodies, he took the latter to be conidia themselves. Since the German investigator, owing very probably to the greater limitations of the microscopes then available, failed to see the highly transparent adhesive substance by means of which the animals were held fast, his account was phrased in most cautious yet under the circumstances quite justifiably non-committal words: "Merkwurdiger Weise geht, soweit meine Beobachtungen reichen, die Infektion stets von der Conidie aus. Sie legt sich an den Wurm an, ..." None could have realized better than he that the application of a non-detachable globose body, whether conidium or other structure, to an animal as active as a nematode, could not have results very serious for the animal unless in some way the latter was prevented from discontinuing the inimical contact.

It is of historical interest that in failing to see the adhesive substance by means of which the supposed conidia of his *Monosporidium repens* held fast their prey, Zopf missed a clue which might well have led to a truer explanation of the efficacy of the anastomosing hyphal loops of *Arthrobotrys oligospora.* In many cases, to be sure, these loops come to enwrap the struggling nema-

[1] Evidently the same fungus was described later from rabbit dung in Bohemia by Bubak as *Monacrosporium leporinum* (Ann. Myc. **4**: 120–121. 1906).

tode with such evident security that the capture appears purely one of mechanical involvement. In numerous other cases, however, the animal is held very close to its oral region where in many species the forward tapering of the body is so marked that extrication would appear to ensue from even a slight backward movement. Or, again, the body of a captured nematode is in contact with the inner surface of the loop over only a small segment of its circumference, so that the animal can not properly be said to be insnared at all. Examination immediately after the moment of capture ordinarily yields no explanation as to why the violent struggles of the animal should be so ineffectual. After some time, however, a coating of colorless, transparent, mucilaginous material of manifestly strongly adhesive properties becomes visible about the areas of contact between hyphal loop and nematode, and continues to increase in thickness and to spread in extent as the struggles are maintained, until in the end it attains considerable volume.

Although on microscopic examination the anastomosing hyphal loops of *Arthrobotrys oligospora* previous to the capture of nematodes do not reveal any coating of adhesive material, the behavior of the animals in coming in contact with these structures provides excellent indirect evidence that such substance is present. The animals show no alarm or embarrassment in brushing ordinary hyphae as they make their way over the surface of agar plate cultures containing mixtures of various fungi, but on touching a hyphal loop they draw back with a suddenness and violence hardly equalled by a person touching a hot stove with his hand. Through this energetic reflex the animals escape capture, in perhaps nine cases out of ten, so that the enormous numbers that are taken and destroyed daily by the fungus in a petri dish culture yet represent only a relatively small proportion of the encounters that occur between nematodes and organs of capture. As might be expected the same reflex is evident in the behavior of nematodes in agar cultures of the various other fungi in which the apparatus of capture likewise consists in whole or in part of an anastomosing system of hyphal loops produced on the surface of the substratum, and in which colorless transparent adhesive material similarly becomes visible following its effective intervention. Among these fungi are included not only most other forms with 1-septate spores

borne in capitate or loosely capitate arrangement, which even when the arrangement is not repeated at successive nodes, would seem more correctly assignable to *Arthrobotrys* since Matruchot (**11**) and later Elliott (**8**) have shown that the mode of spore formation in *Cephalothecium roseum* Corda (= *Trichothecium roseum* Link), the type species of the genus *Cephalothecium,* is fundamentally different, but also at least one species with swollen, typically 3-septate spores assignable evidently to *Dactylaria* (**4**, *fig. 5, A*) and another with swollen, 3-septate or 4-septate spores (**6**, *fig. 12, A*) which appears eligible for inclusion either in *Monacrosporium* or in *Dactylella.*

As in the fungus last referred to the superficial hyphal loops arise by the development of bridging connections between rather regularly spaced, short, bristling, mostly 2-celled processes (**6**, *fig. 12, B*), which are adhesive more especially on the distal cell, the performance of organs of capture corresponding substantially to the type illustrated by Zopf for his *Monosporidium repens* and to the type exemplified in *Arthrobotrys oligospora* can here be observed side by side. It now becomes apparent that the stubby processes are effective mainly in the capture of the younger, smaller animals, whereas the closed loops by restricting the movements of the prey, and especially by engaging it over a much more extensive adhesive surface, are adapted to hold even the fully grown vigorous adults. It was no mere coincidence therefore that Zopf found his *Monosporidium repens* destructive to " eine auffallend kleine und schmale . . . Anguillulide." Similarly in agar plate cultures captures on the adhesive knob-cells of the probably identical fungus which from the morphology of its conidia would seem the same as *Dactylella ellipsospora,* were found restricted to the smaller individuals of the species of *Rhabditis, Cephalobus* and *Diplogaster* present. The considerably smaller structures represented in the globose cells produced by the fungus provisionally identified as *Dactylaria candida* could therefore hardly be expected to retain any but the smallest larvae, and that perhaps only in the absence locally of firm material providing leverage for the struggling prey. Their usual inefficiency as organs of capture in agar culture media consequently need not imply any lack of effectiveness

in the various materials of far different texture which constitute the field of predacious activity in nature.

Whether adhesive material is present on the solitary non-constricting loops which Sherbakoff interpreted as conidia, remains somewhat uncertain. None has been observed, but as these organs in both of the species known to produce them are formed within the agar instead of on the surface, the increased optical difficulties might possibly account for this failure. Yet altogether apart from considerations based on direct visual evidence, the performance of these organs is not such as to indicate any necessity for prolonged participation by adhesive material. In all cases of capture they soon fit very snugly around the insnared prey, generally, indeed, becoming jammed so tightly on the tapering body of the struggling animal that the integument is noticeably indented at all points along the circumference of contact (7, *fig. 16, C*).

The same optical difficulties intervene in the examination of the constricting loops formed in some predacious representatives of the genera *Trichothecium* (4, *fig. 10*), *Arthrobotrys* (6, *fig. 13*), *Dactylaria* (6, *fig. 14*) and *Monacrosporium* (7, *fig. 17*). In none of these representatives has adhesive material been observed, and obviously such substance could here be of no possible use except perhaps for a brief period immediately following capture. Once the swelling of the three component cells is under way, the constricting loop reveals itself as a powerful compressing mechanism as remarkable in adaptation to its special function as it is conspicuous among devices employed by carnivorous plants for the impression of unrelenting malevolence it conveys.

In any case, however, the utilization of adhesive material on the organs of many of the nema-capturing Hyphomycetes provides an important parallelism between this group of predacious forms and the various predacious Phycomycetes that have become known, including the two nema-capturing species figured earlier (4, *fig. 8*; 6, *fig. 15*). In these two fungi the adhesive substance is apparently of a different composition from that common to the several Hyphomycetes, as it very soon assumes a deep golden yellow color which makes it very readily visible under the microscope. Its adhesiveness too would seem to be markedly stronger, as in the form bearing large obovoid conidia on tall erect conidiophores (4, *fig. 8, A*),

nearly fully grown nematodes are held securely. A bulbous expansion broadly fused to the animal here usually puts into appearance (4, *fig. 8, C*), but as its development apparently occurs after capture of the nematode and preceding perforation of the integument, it seems probable that its function may be more directly related to the latter event than to the former. Both in this fungus and in the nema-capturing fungus which appears referable to the genus *Pythium* Pringsh. (6, *fig. 15*) external mycelial modification at the time and place of capture is absent or so inconsiderable that one can hardly speak of special organs. The sigillate outline so characteristic of the cushion of adhesive material attaching the prey to the *Pythium* filament is, of course, not to be interpreted as a result of morphological differentiation. It merely indicates rather that the fashioning of the cushion is closely comparable to the fashioning of a wax seal which it simulates, the struggling animal evidently exerting on the mass of plastic adhesive material a pressure analogous to the pressure of a stamp on heated wax. Yellow adhesive material of similar appearance and consistency is effective in the capture of amoebae by a series of unusually delicate Phycomycetes, which as has been set forth, bear distinctive aerial conidia and small intramatrical sexual organs (5, *figs. 2–5*).

Possibly the general similarity in manner of taking prey furnishes a slight indication of taxonomic affinity between the delicate amoeba-capturing Phycomycetes and the much sturdier nema-capturing form with large obovoid conidia. That the latter fungus is not a lone representative of its type has recently become evident through the discovery of several undoubtedly congeneric nema-capturing forms, which more frequently come to bear a number of conidia on a conidiophore, following the repeatedly continued growth of the supporting stalk below each successive terminally formed reproductive body. Whether the large non-septate aerial conidia of these forms can be homologized with the submerse and even larger gemmae which Arnaudow (2) described as being produced by the aquatic rotifer-capturing *Zoophagus insidians* Somm. remains uncertain for the present. If analogy is not misleading, the diversity in apparatus of capture found among the fairly closely interrelated Hyphomycetes predacious on nematodes would in-

dicate that the production of the somewhat specialized organs of capture in the short adhesive branches described by Sommerstorff (**15**) and by Arnaudow (**1**) in the original accounts of *Z. insidians* and the similarly aquatic, rotifer-capturing *Sommerstorffia spinosa* Arn. respectively, need not preclude relationship to forms without such predacious modifications.

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THE STRUCTURE AND DEVELOPMENT OF A NEW AQUATIC PHYCOMYCETE [1]

ARTHUR G. KEVORKIAN

(WITH 11 TEXT FIGURES)

Among the aquatic Phycomycetes the genus *Araiospora* of the family Leptomitaceae was first established by Thaxter in 1896 upon the single species *A. pulchra*. The genus is readily distinguished by the presence of two types of sporangia, one subcylindric with thin, smooth wall, the other obpyriform with thick, spinose wall, and by the formation within the oögonium of a periplasmic layer of hexagonal appearing cells surrounding the central oöspore. To this newly erected genus Thaxter at the same time (**1896**) transferred Cornu's *Rhipidium spinosum* (**1872**) because of its spinose thick-walled "secondary" sporangia, calling this new combination *A. spinosa*. It remained for von Minden (**1915**, **1916**), however, to add to our knowledge of this scantily described species, for from material collected and studied in Germany he described in detail the developmental stages not only of the sporangia but also of the sexual organs hitherto unknown. Since the two foregoing species were both characteristic of temperate regions, Linder's (**1926**) description of a new species from British Guiana is of interest, for it is the first instance in which a member of the genus *Araiospora,* or indeed a representative of the entire family, has ever been reported from the tropics. This species, *A. coronata,* is unique in that the 4 to 6 spines about the apical exit papilla of the "secondary" sporangia are 7.9 μ in length, whereas in *A. spinosa* the spines are more numerous and much longer (60–70 μ). Although the sexual organs were not found in Linder's species, there was ample basis for his establishing it as new since in addition to the distinctive number and arrangement of the spines the sizes of the various organs differed from those of the already established species (see TABLE 1).

[1] Contribution from the Cryptogamic Laboratories of Harvard University No. 125.

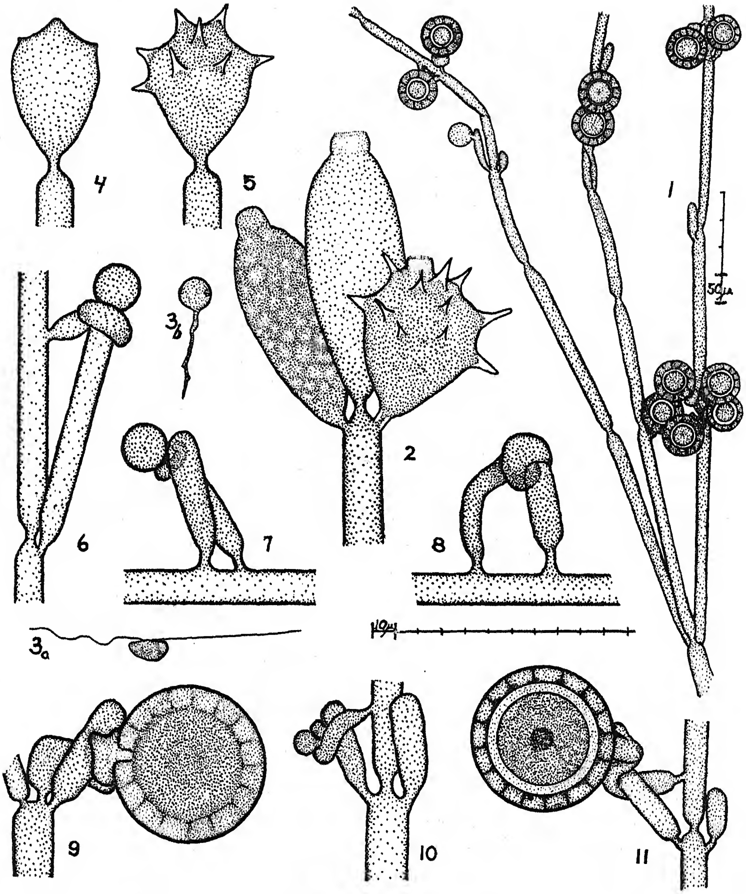

Fig. 1–11. All drawings were made from material mounted in lacto-phenol and cotton blue and were drawn with the aid of a camera lucida with 10 × ocular and 4 mm. objective, with the exception of figure 1, which was drawn with 10 × ocular and 16 mm. objective. Each division of the scale accompanying figure 1 equals 50 μ; of the other scale, which applies to the remaining figures, each division equals 10 μ. 1, Portion of a plant showing the arrangement and grouping of the sexual organs. × 65; 2, A terminal portion of a sporangiophore showing the smooth and spinose types of sporangia. The spinose and one of the smooth sporangia have already discharged their spores. × 350; 3a, A typical laterally biciliate zoöspore, 3b, Germination of zoöspore by means of a germ tube which later becomes a much branched rhizoidal system. × 350; 4–5, Stages in the development of the spines of the spinose type of sporangium. × 350; 6, 7, 8, and 10, Early stages in the development of the sexual organs, figures 6 and 10 showing the origin of the antheridium and oögonium from two adjoining

Since this genus so far has comprised but these three species, of which only two are known to show sexual reproduction, the finding of an additional sexually reproductive species with antheridia characteristically and distinctively twined about the base of the oögonia seems to justify bringing it to the attention of mycologists in the present note. This organism, which for reasons appearing later in this paper will be called *Araiospora streptandra,* was found growing saprophytically on submerged twigs of *Prunus* and *Salix* in ponds and ditches around Rhode Island State College, Kingston, R. I., and near Cambridge, Massachusetts, during the months of April and May in 1931, 1932, and 1933. Gross cultures were perpetuated by transferring selected portions of twigs infected with the desired fungus but relatively free from other organisms, to cooled mason jars partially filled with sterile distilled water, adding twigs of *Acer* and *Prunus* from time to time to support new growth, and changing the water frequently to offset the accumulation of bacteria and other decomposing agents. It would, of course, have been desirable to grow this fungus in pure culture on artificial media as von Minden (**1916**) successfully cultured *Araiospora spinosa* and *Rhipidium europaeum,* but unfortunately the writer, like others (Kanouse, **1927**, p. 290), was unsuccessful in attempts even though he followed von Minden's methods most carefully and also tried numerous variations of it. Yet even though pure cultures were not obtained, the gross cultures mentioned above allowed the fungus to complete its life cycle apparently normally under conditions approximating those in nature.

Under these conditions the fungus was studied in its various stages and found to show the following structure and development.

DEVELOPMENT OF THE FUNGUS

Thallus. The zoöspore of *Araiospora* upon germination gives rise to a slender germ-tube (FIG. 3b) which in turn branches into

segments of the mycelium, whereas figures 7 and 8 show the origin of the sexual organs from the same segment. $\times$ 350; 9, Later stage in the development of the sexual organs, showing irregularly outlined antheridium with fertilization tube, and oögonium differentiating into peri- and oöplasm. $\times$ 350; 11, More advanced stage of the oögonium showing, in optical section, the peripheral layer of hexagonal-appearing cells surrounding the thick-walled central oöspore. $\times$ 350.

a definite rhizoidal system extending into the tissues of the substratum. In the meantime the zoöspore body elongates and develops into a greatly enlarged, sub-cylindrical, basal segment in a fashion comparable to that described in detail by Linder (**1926**) for *A. coronata.* From the sub-conical apex of this basal segment, are borne many repeatedly and umbellately branched filaments, which are constricted at regular intervals, each successive member becoming more elongate and slender than its predecessor, the plant thus presenting an arbusculate appearance (FIG. 1), typical of the higher Leptomitaceae and the Blastocladiaceae.

Sporangia. The two types of sporangia found in the other species of the genus *Araiospora* are present in this species also. Both of these, the smooth, thin-walled, sub-cylindrical to broadly clavate type (FIG. 2), and those which are thick-walled, broadly oval, pyriform, and spiny (FIG. 2, 4, 5), are borne in whorls of 2 to 6 at the distal ends of the segments.

The thin-walled smooth sporangium is very similar to that of the genus *Sapromyces,* but the subsequent formation of spinose sporangia separates the two rather closely related genera. These sporangia appear as small, knob-like bodies separated from the rest of the mycelium by constrictions which are elongated, heavy-walled, collar-like modifications of the hyphal walls (FIG. 2). The protoplasm which flows in from adjoining segments passes through the narrowed openings and causes the sporangium to elongate, until finally the contents of the large (79–111 $\mu \times$ 29–49 μ), sub-cylindrical body begins to differentiate into zoöspores, whereupon the lumen of the constriction is obliterated by the additional centripetal thickening of the wall at the constriction.

Save for the thicker walls, the spinose sporangia (60–78 $\mu \times$ 46–63 μ) resemble the smooth type in their early stages. A little later in their development, however, small protuberances begin to appear at the apical portion of the sporangial wall (FIG. 4) and develop in basipetal succession (FIG. 5). When mature, the 10 to 15 elongate conical spines (15 to 30 μ in length) are scattered over the surface of the sporangium in a regular manner, as in *A. pulchra* Thaxter.

Both types of sporangia show great similarity, not only in the early stages of development described above, but also in the subse-

quent formation of zoöspores. The scattered median vacuoles increase in size and coalesce until they occupy a large central area. A decided swelling of the sporangium follows, accompanied by changes in texture of the protoplasmic content, and the first signs of zoöspore differentiation become apparent. The zoöspore initials next make their appearance as densely granular, somewhat angular masses filling the entire sporangium, and when completely differentiated are discharged through an apical modification of the sporangium wall, the papilla of dehiscence. Upon emergence the zoöspores linger about the mouth of the sporangium for a few seconds, until they have assumed their typical, somewhat kidney-shaped, laterally biciliate form (FIG. 3*a*), and then with a few twitching movements orient their cilia and swim away. Their activity is monoplanetic, for after a period of swarming about they lose their cilia, come to rest, round up, and later germinate by giving rise first to a rhizoidal system and subsequently to the elongated enlarged basal cell as already described.

Sexual organs. The sexual organs, which develop some time after the sporangia and which in the material collected were usually found more abundantly in the spring, consist of large spherical oöspores surrounded by a layer of peripheral cells which in surface view are hexagonal and borne on short lateral branches, either singly or in clusters of two to four (FIG. 1, 11) near the distal ends of the hyphal segments; and of antheridia, which are irregular in shape, borne on similar branches near the oögonia, twisted about the constriction separating the oögonium from the stalk cell (FIG. 10).

Antheridia. The antheridia develop as short lateral branches arising usually near the oögonial initials (FIG. 7, 8), less commonly at some distance (FIG. 6), and twining around the oögonial stalk as in *Pythium mastophorum* Drechsler (**1930**) and other related species. When mature, the terminal portion of the antheridium, swollen and somewhat irregularly lobed, is attached to the base of the oögonium close to its juncture with the stalk. From the mature antheridium a slender fertilization tube penetrates the oögonial wall without indenting it (FIG. 9), and extends some distance into the oösphere, as described by Thaxter (**1896**) in the case of *A. pulchra.* This is contrary to the description of King

(1903), who, on the basis of cytological evidence in that species, interpreted this fertilization tube as formed by the periplasm of the oögonium just previous to fertilization. Cytological observations upon this new *Araiospora,* to be undertaken later, will, it is hoped, ultimately settle this disputed point. A large portion of the antheridial protoplasm is next discharged into the oösphere by the rupturing of the tip of the fertilization tube, leaving the antheridium vacuolate, or even empty.

Oögonia. The oögonia first appear as small, knob-like projections (FIG. 6, 7) borne singly or in clusters on short lateral branches from which they are separated by constrictions. Early in the development of the oögonia the antheridia become applied to the oögonial walls. As the oögonium matures, the finely granular, homogeneous texture of its content gradually becomes coarsely and more densely granular in the central portion, while the periphery remains unchanged, thus differentiating a dense central oöplasm surrounded by a peripheral area of finely granular periplasm. At maturity the spherical oögonium, 52 to 68 μ in diameter, and with hyaline to yellowish wall 3 to 6 μ thick, contains a peripheral zone of several more or less hexagonal-appearing peripheral cells (8 to 12 μ in diameter) of periplasmic origin, surrounding the single central oöspore of oöplasmic origin, 39 to 46 μ in diameter, with a dense content including a single central oil mass, and with a heavy wall 8 to 10 μ thick.

RELATIONSHIP AND IDENTITY

Araiospora streptandra, although rather closely related to *A. pulchra,* is readily distinguishable because its peculiarly twisted, usually unbranched, irregular antheridium is borne as a short lateral branch near the distal end of any given segment and applied at the base of the laterally borne oögonium, whereas in *A. pulchra* the antheridial branches are terminal, usually recurved, and applied, without twisting or turning, to the bases of terminally borne oögonia. Moreover, the androgynous antheridium and the numerous scattered spines of this new species, in contrast to the diclinous antheridium and apically located spines of the "secondary" sporangium of *A. spinosa,* readily separate *A. streptandra* from this latter species.

In addition to these outstanding qualitative characters which distinguish this new form, there are certain quantitative differences in the size of important organs, as is shown in the following comparative table.

TABLE 1

Organism	Smooth Sporangia	Spiny Sporangia	Spines	Oöspores	Oögonia
Araiospora pulchra	120–175 × 30–35μ	48–70 × 45–60μ	10–35μ	35–45μ	50–60μ
A. spinosa	90–150 × 45–60μ	100–150 × 40–80μ	60–70μ	Measurements not given	
A. coronata	63–85 × 11.5–16.2μ	68–130 × 12–26μ	7–9μ	Not known	
A. streptandra	79–111 × 29–49μ	60–78 × 46–63μ	15–30μ	39–46μ Av. 44–46μ	52–68μ

It is obvious from the characteristic arrangement of the sexual organs, together with various dimensional differences presented in table 1, that this is a species hitherto undescribed. Its diagnosis therefore is given as follows:

DESCRIPTION

Araiospora streptandra sp. nov. Large sub-cylindrical basal cell with many branches arising from the sub-conical apex. Branches separated by constrictions and repeatedly and umbellately branched, each successive segment becoming more elongate and slender than its predecessor. Sporangia borne singly or in whorls of two to six, of two types (1) sub-cylindric or broadly clavate and smooth, 79–111 × 29–49 μ, (2) oval or pyriform and spiny, 60–78 × 46–63 μ. Spines numerous, 15 to 30 μ in length, elongate conical in shape. Antheridia borne singly on short, stout lateral branches, usually originating near the distal ends of the segments, twisted about the base of the oögonia, irregular in outline. Oögonia spherical, 52–68 μ (Av. 60–64 μ), arising similarly to and usually near the antheridia. Oöspore spherical, 39 to 46 μ (Av. 44 to 46 μ), surrounded by a single layer of hexagonal-appearing

peripheral cells derived from the periplasm. Germination of the oöspore not observed.

On submerged twigs of *Prunus* and *Salix,* in the vicinity of Rhode Island State College, Kingston, Rhode Island, and of Cambridge, Massachusetts.

Type material deposited in the Farlow Herbarium.

Cellula fundamentale magna, sub-cylindrata, ramis densis ex apice sub conico orientibus. Ramis identidem constrictis et racemose diffusis quorum segmenta longiora angustioraque gradatim fiunt. Duorum exemplorum altero sporangio subcylindrico et leve (79–111 × 29–49 μ) in apice tumescente; altero spinis numerosis (15 to 30 μ) aut ovato aut forma piro simile (60–78 × 46–63 μ) singulo aut numero ad sex in orbem consistentes ex extremis segmentis aut ex extremo segmento singulo. Antheridiis forma inconstante singulariter orientibus ex lateralibus ramis brevibus et robustis plerumque prope extrema segmenta ulteriora quae circum basim oogoniarum se torquent. Oogoniis globosis, 52–68 μ (av. 60–64 μ), in modo antheridiorum orientibus quibus proxima sunt. Oosporis globosis strato cellularum sexangularum ex periplasma deductorum circumdatis. Germinatio oosporarum non observata.

Hab. In ramulis *Pruni* et *Salicis* submersis ad Rhode Island State College, Kingston, Rhode Island et Cambridge, Massachusetts.

In conclusion the writer wishes to acknowledge his indebtedness to Professor W. H. Weston, Jr., for his helpful criticism and constant interest, and to Dr. David H. Linder for advice and for material of *Araiospora coronata.*

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NEW GENERA AND SPECIES OF LICHENS FROM THE HERBARIUM OF BRUCE FINK. II.[1]

JOYCE HEDRICK[2]

1. **Synechoblastus wyomingensis** Fink, sp. nov.

Transformans hospitalem algam in corpus irregulare, ascendens multilobatum, leve, obscure olivaceum et nigrescens, lobis numerosis, brevibus, integris aut sinuosis, infra levibus et pallidibus; apothecia parva, 0.75–1.4 mm. lata, sessilia in marginibus loborum, disco plano vel leviter convexo, rufescenti-fusco et obscuriore, excipulo algoideo prominenti, integro, concolore thallo; sporae 4 rare 8, decolores, ellipsoideo-acutae et interdum extentae in appendicem 3–5 μ longam, 3-septatae, 22–32 × 6.5–9.5 μ.

Transforming the algal host into a small, thin, irregular, ascending, much lobed, smooth, dark olive green and blackening body, the lobes numerous, short, entire or sinuose; smooth and lighter colored below; apothecia small, 0.75–1.4 mm. across, sessile along the margins of the lobes, the disk flat to slightly convex, reddish brown and darker, the algoid exciple prominent, entire, colored like the thallus; hypothecium hyaline; hymenium hyaline below and brownish above; paraphyses thick, septate, hyaline below and brownish toward the rarely branched apices; asci clavate; spores 4—rarely 8, hyaline, ellipsoid-pointed and occasionally extended into an appendage 3–5 μ long, 3-septate, 22–32 × 6.5–9.5 μ.

The algal host is *Nostoc*.

On limestone cliff east of Laramie, Wyoming, collected by Edwin B. Payson, No. *4233*, November 16, 1924 (type).

Similar to *S. laciniatus* (Nyl.) Fink, but the lobed thalloid body somewhat smaller and the algoid exciple more prominent. The spores larger than in *S. laciniatus,* and the pointed end sometimes extending into an appendage which would separate it from all other species known from the United States.

2. **Collema fayettense** Fink, sp. nov.

Transformans hospitalem algam in corpus parvum et tenue vel crassiusculum, irregulare lobatum, viridescens vel viridi-fuscum, lobis levibus

[1] No. 1 of this series appeared in Mycologia **25**: 303–316. 1933.

[2] Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 434.

vel leviter rugosis, demum plus minusve imbricatis, adscendentibus ad marginem crenatis; infra pallidioribus et saepe minute rugosis, hyphis saepe protrudentibus in minutis areis pleremque marginalibus; apothecia minuta vel parva, 0.1–0.3 mm. lata, numerosa, immersa, disco concavo vel fere plano, fusco, excipulo algoideo leviter crasso et prominenti; sporae 8, decolores, oblongae vel ellipsoideo-acutae, 3–4 transverse septatae et 1 longitudinal septatae, 18–22 × 9–11 μ, inconditae.

Transforming the algal host into a small, thin to somewhat thick, irregularly lobed, greenish to greenish brown body, the lobes smooth to somewhat wrinkled, becoming more or less imbricated, with crenate, ascending margins; lighter below and often minutely wrinkled, the hyphae often protruding in minute whitish areas commonly along the margin; apothecia minute to small, 0.1–0.3 mm. across, numerous, immersed, the disk concave to almost flat, brown, the algoid exciple rather thick and prominent; hypothecium hyaline or tinged brownish; hymenium hyaline; paraphyses more or less coherent and indistinct; asci long-clavate; spores 8, hyaline, oblong to ellipsoid-pointed, 3–4-septate transversely and 1-septate longitudinally, 18–22 × 9–11 μ, irregularly arranged.

The algal host is *Nostoc*.

On exposed limestone near Big Rock, Fayette, Iowa, collected by Bruce Fink in 1896 (type), Fink Herb. No. *15, 482*.

Similar to *C. plicatile* Ach., but the margins of the lobes are not plicate, the apothecia are much smaller and the spores are shorter.

3. **Collema pustulatum heterosporum** Fink, subsp. nov.

Sporae oblongo-ellipsoideae, una extremitate subplanae, 3–5 transverse septatae et 1–3 longitudinale septatae, aut rare sphaeroideae et 3–5 septatae transverse et longitudinale, 25–28 × 13–14 μ, inconditae aut rare uniseriatae.

Spores oblong-ellipsoid with one end slightly flattened, 3–5-septate transversely and 1–3-septate longitudinally, or rarely sphaeroidal and 3–5-septate transversely and longitudinally, 25–28 × 13–14 μ, irregularly or rarely uniseriately arranged.

On rocks in Florida, collected by Britton and Britton (type), Fink Herb. No. *15, 483*.

4. **Leptogium perminutum** Fink, sp. nov.

Transformans hospitalem algam in corpus parvum, tenue, rugosum et inaequale, tenaciter adnatum, nigrum, plus minusve intectum ramulis minutis et coralloideis, cortice plectenchymatico; apothecia minuta, 0.1–0.3 mm. lata, adnata vel sessilia, rotundata, numerosa, dispersa aut aggregata, disco concavo vel plano, fuscescento vel rufescenti-fusco vel fuscescenti-nigro, madido

lucide rufescenti-fusco, excipulo algoideo tenui vel crassiusculo, levi vel rare leviter confragoso aut crenato; sporae 8, decolores, ellipsoideae vel acuto-ellipsoideae, 5 transverse septatae et 1–3 longitudinale septatae, loculis fere cubicis, 18–25 × 8–10 μ, inconditae.

Transforming the algal host into a small, thin, wrinkled and irregular, closely adnate, black, crust-like body, more or less covered with minute coralloid branchlets, the cortex plectenchymatous; apothecia minute, 0.1–0.3 mm. across, adnate to sessile, round, numerous, scattered or clustered, the disk concave to flat, brownish to reddish brown or brownish black, bright reddish brown when moistened, the algoid exciple thin to moderately thick, smooth to rarely somewhat rough or wavy; hypothecium hyaline; hymenium hyaline to yellowish; paraphyses unbranched, distinct and parallel or becoming conglutinate; asci clavate; spores 8, hyaline, ellipsoid to pointed-ellipsoid, 5-septate transversely and 1–2-septate longitudinally, the cells almost cubical, 18–25 × 8–10 μ, irregularly arranged.

The algal host is *Nostoc,* found in short chains.

On old wood in woods near Oxford, Ohio, collected by Bruce Fink, May 1927 (type).

The thalloid body similar to that of *L. rhyparodes* Nyl. and *L. schraderi* (Bernh.) Nyl., but perhaps more like that of *L. tenuissumum* (Dicks.) Fries, but less covered with apothecia; the apothecia smaller and the spores much smaller.

5. **Lecidea congesta** Fink, sp. nov.

Thallus parvis, rotundatis vel difformis saepe asper dispersis verrucis, virescenti-glaucis vel sordide fuscis vel obscuris; apothecia parva vel mediocria, 0.4–1.8 mm. lata, adnata, disco convexo, pallido vel obscuro fusco, excipulo tenui, cincto velo thalli tenuissimo, solum conspicuo ad margines basales hymenorum novellissorum, et extemplo evanescente, apothecia matura demum difformia et sulcata, supra 1–4 vel rare pluribus sulcis extentis in varias partes, primum vadosis demum diffindentibus apothecium in 2–5 partes multiformes, sicut apothecia plura et difformia conglomerata; spores 8, decolores, non septatae, oblongo-ellipsoideae vel ellipsoideae, 9.5–11 × 4.5–5 μ.

Thallus of small, round to slightly irregular, often scattered, greenish gray to dirty brown or darker warts; apothecia small to middle-sized, 0.4–1.8 mm. across, adnate, the disk slightly to strongly convex, light to darker brown, the exciple thin, surrounded by a very thin thalloid veil, exciple and veil obscurely visible at the basal margins of very young hymenia, but disappearing very early, the mature apothecia becoming variously irregular in form and furrowed above with 1–4 or rarely more furrows running in va-

rious directions sometimes intersecting, at first shallow, but finally splitting the apothecium into about 2–5 variously shaped, closely-placed portions, giving the appearance of as many peculiarly shaped, conglomerate apothecia; hypothecium and hymenium hyaline to pale yellowish; paraphyses stout, somewhat gelatinized and semidistinct, rarely branched above, enlarged toward the apices where sometimes tinged brownish; asci broadly clavate and sometimes saccate; spores 8, hyaline, non-septate, oblong-ellipsoid to ellipsoid, 9.5–11 × 4.5–5 μ.

The algal host is *Protococcoid.*

On a granite boulder in an open pasture near Eaton, Montgomery County, Ohio, collected by Bruce Fink, No. *247,* April 10, 1914 (type), Fink Herb. No. *7980.*

Belonging to the *Biatora*-like section of *Lecidea* with soft and light-colored hypothecium. Similar to *L. sylvicola* Flot., but easily separated by the larger and deeply furrowed apothecia and the larger spores.

6. **Bilimbia Pammellii** Fink, sp. nov.

Thallus granulosus, contiguus vel dispersus, virescenti-glaucus, formans tenuem crustam rimosam; apothecia parva vel mediocria, 0.3–0.7 mm. lata, adnata, dispersa vel rare aggregata, disco convexo, nigro, excipulo concolori et mox exanescente; sporae 8, decolores, oblongo-ellipsoideae, 3 septatae, 15–24 × 3–4.5 μ.

Thallus granulose, continuous or scattered, greenish gray, becoming a thin, chinky crust; apothecia small to middle-sized, 0.3–0.7 mm. across, adnate, scattered or rarely clustered, the disk slightly to strongly convex, black, the exciple of the same color and soon disappearing; hypothecium pale brown; hymenium hyaline; paraphyses unbranched, somewhat thickened at the apices; asci clavate; spores 8, hyaline, oblong-ellipsoid, 3-septate, 15–24 × 3–4.5 μ.

The algal host is *Protococcoid.*

On sandstone at The Ledges, Boone County, Iowa, collected by Bruce Fink, July 27, 1903 (type), Fink Herb. No. *6680.*

Similar to *B. sphaeroides* (Dicks.) Koerb. and *B. epixanthoides* (Nyl.) Lettau, but differing from the latter which has a powdery yellowish thallus and from the former in the slightly heavier thallus and the smaller apothecia with black disk.

7. **Cladonia cristatella densissima** Fink, subsp. nov.

Squamae quam in f. typica crassiores, minores et minus lobatae, demum imbricatae in 3–4 ordines; podetia abortiva vel brevissima, rare plus quam 3–4 mm. longa, dense squamulosa, squamis infimis magnitudine communibus, superioribus minoribus et supernis interdum verruciformibus; apothecia minuta, 0.1–0.4 mm. lata, plerumque aggregata in apice podetiorum, sed nonnumquam in lateribus er interdum in squamis thalli.

The squamules thicker, rather smaller and less lobed than in the species, closely packed and becoming imbricated in 3 or 4 layers; podetia abortive or very short, scarcely surpassing 3 or 4 mm. in length, densely covered with squamules, the basal ones of ordinary size and the upper ones reduced and sometimes passing into wart-like bodies toward the apex; apothecia minute, 0.1–0.4 mm. across. mostly grouped at the apex of the podetia but sometimes occurring on the sides as well and sometimes even seated on the squamules of the primary thallus.

On top of post in dry pasture, near Oxford, Ohio collected by Bruce Fink, March 30, 1927 (type), Fink Herb. No. *15,494*.

8. **Cladonia Herrei** Fink, sp. nov.

Thallus primarius ex squamis parvis vel mediocribus constans, plerumque elongatis et demum aliquoties alte lobatis, plerumque ascendentibus, planis vel leviter involvatis, aggregatis vel dispersis et interdum evanescentibus, viridule glaucis vel umbrinis, lobis saepe crenatis; infra albidis; podetia in squamis primarii thalli formata aut in podetiis morientibus, KOH + (suffusca), longa et gracilia, erecta aut ascendentia, cylindrica, pluries subdichotome ramosa, squamis destituta aut plus minusve squamosa, interdum ommino, squamis sursum multo minoribus et rotundatis, vix lobatis, cortex subcontinuus vel demum dispersus aut confragosus et subareolatus, areolis continuis aut demum rare subdispersis, lateribus et axibus rare perforatis, sterilibus apicibus furcatis, acutis in spinulam desinentibus, interdum perforatis, viridule glaucis vel olivaceo-fuscis, rare scyphiferis; scyphi parvi; apothecia parva, 0.3–0.6 mm. lata, in vel sub terminos ramorum obtusorum aut rare in marginibus scyphorum, saepe aggregata aut conglomerata, disco valde convexo vel subsphaeroideo, pallido vel obscure fusco aut demum nigricante; sporae 8, decolores, non septatae, ellipsoideae, 8–11 $\times$ 2.5–3.5 μ.

Primary thallus composed of small to middle-sized, usually elongated and finally several times deeply lobed, commonly ascending, flat to slightly inward-rolled, clustered or scattered and sometimes disappearing, greenish gray to brownish squamules, their lobes often crenate; whitish below; podetia arising from the squamules of the primary thallus or from dying podetia, KOH + (brownish), long and slender, erect or ascending, subdichotomously

much spreading branched, without squamules or more or less squamulose, sometimes throughout, the upper squamules much smaller and almost round, with little or no lobing, the cortex subcontinuous to chinky or rough and subareolate, the areoles continuous or finally and rarely somewhat scattered, the sides and axils rarely perforate, the sterile tips forked and spinous pointed, sometimes perforate, greenish gray to olive brown, very rarely cup-bearing; cups small; apothecia small, 0.3–0.6 mm. across, on or below the ends of the obtuse branches or very rarely on the margins of the cups, commonly clustered or conglomerate, the disk strongly convex to subspherical, light to darker brown, or finally blackish; hypothecium hyaline; hymenium hyaline below and brownish above; paraphyses hyaline, unbranched or branched toward the somewhat enlarged brownish tips; asci clavate with the apical wall thickened; spores 8, hyaline, ellipsoid, non-septate, $8–11 \times 2.5–3.5\ \mu$.

The algal host is *Pleurococcus*.

In crevices of rocks in the foothills of the Santa Cruz Mountains, California, collected by A. C. Herre, August 14, 1903, Fink Herb. No. *6502* (type) and No. *6631*.

Doctor Herre published this as *Cladonia furcata racemosa* (Hoffm.) Floerke, in Proc. Wash. Acad. Sci. **7**: 391. 1906. Similar to *C. subsquamosa* (Nyl.) Vainio but without the characteristic powdery-squamulose surface of that species. Note in Prof. Fink's handwriting on the herbarium packet reads, " Scriba says hardly *C. subsquamosa;* the surface similar to *C. degenerans lepidota* Nyl." The last named subspecies has been placed by Vainio under *C. gracilescens* (Floerke) Vainio. Though the surface is similar, the plant is much smaller. It is also similar to *C. rangiformis* Hoffm., but usually smaller and squamulose.

9. **Acarospora immersa** Fink, sp. nov.

Thallus tenuis, levis vel minute rimosus et areolatus, obscure viridi-glaucus vel niger; apothecia minuta, 0.1–0.15 mm. lata, immersa, solitaria aut rare plura in areola, disco plano, canescenti-pruinoso, excipulo tenui et concolori thallo, integro; sporae numerosae, decolores, ellipsoideae, non septatae, $3–4 \times 1.5–2\ \mu$, inconditae.

Thallus thin, smooth to minutely chinky and areolate, dark greenish gray to black; apothecia minute, 0.1–0.15 mm. across, immersed 1 or rarely more in an areole, the disk flat, grayish pruinose, the exciple thin, colored like the thallus, entire; hypothecium and

hymenium hyaline; paraphyses hyaline, coherent and semidistinct; asci clavate and becoming inflated; spores numerous, hyaline, ellipsoid, non-septate, 3–4 × 1.5–2 μ, irregularly arranged.

The algal host is *Protococcus*.

On limestone in grassy, open pasture near Oxford, Ohio, collected by Bruce Fink, May 15, 1927 (type).

Similar to the one specimen (Fink Herb. No. *8888*, identified by A. Zahlbruckner) of *A. heppii* Naeg. seen from the United States, but differing in the pruinose disk of the apothecium and the smaller spores.

10. Acarospora saxicola Fink, sp. nov.

Thallus squamosus, squamis parvis vel mediocribus, difformibus, interdum lobatis, demum imbricatis et formantibus crustam crassam et inaequalem, areolatam, glaucam aut sordide albidam, substrato plus minusve tenaciter adjunctam; apothecia parva vel mediocria, 0.4–1.2 mm. lata, immersa vel adnata, 1 aut plura in areola aut in squama, disco plano vel leviter convexo, fusco vel suffusce nigre aut canescenti-pruinoso, excipulo tenui, concolori thallo, integro vel leviter inaequali et crenulato; sporae numerosae, decolores, sphaeroideae, non septatae, 2.5–4.5 μ lata, inconditae.

Thallus squamulose, the squamules small to middle-sized, irregular, sometimes lobed, becoming imbricated and passing into a thick, irregular, areolate, grayish or dirty white crust, more or less closely attached to the substratum; apothecia small to middle-sized, 0.4–1.2 mm. across, immersed to adnate, 1 or more in an areole or squamule, the disk flat to slightly convex, brown to brownish black or grayish pruinose, the exciple thin, colored like the thallus, entire to slightly irregular and crenulate; hypothecium hyaline or cloudy; hymenium hyaline to brownish above; paraphyses hyaline, unbranched, jointed, becoming somewhat conglutinate; asci clavate, becoming inflated, the wall much thickened especially above; spores numerous, hyaline, spherical, non-septate, 2.5–4.5 μ in diameter, irregularly arranged.

The algal host is *Protococcus*.

On rocks at 5400 feet, Naturita, Montrose County, Colorado, collected by Edwin B. Payson, July 1914 (type).

Similar to *A. Scheicheri* (Ach.) Mass., differing in substratum, in the color of the thallus which also often passes into a thick uneven crust, in the sometimes pruinose disk and the slightly smaller, more spherical spores.

11. **Pertusaria lecanina nigra** Fink, subsp. nov.

Apothecia demum obscure nigra vel nigricanti-pruinosa; sporae 2, decolores, ellipsoideae, 100–128 × 40–50 μ.

Apothecia becoming dull black or blackish pruinose; spores 2, hyaline, ellipsoid, 100–128 × 40–50 μ.

On old yew tree at 3000 feet, Rost Lake, Montana, collected by W. P. Harris, July 15, 1901 (type).

12. **Lecanora bipruinosa** Fink, sp. nov.

Thallus crassus, adnatus, pallide fuscus, disperse albido-pruinosus, parte centrali partim rimosus vel subareolatus aut verrucosus et partim lobatus (distincte ad margines), lobis convexis, plus minusve transversaliter rupto-sulcatis, aliquantum brevibus, marginibus integris vel incomposite et crasse fluctuoso-crenatis, apicibus interdum nigricanti-tinctis; apothecia parva vel mediocria, 0.5–2 mm. lata, subsessilia, disco plano vel leviter convexo, flaventi-viridi-pruinoso, excipulo concolore thallo, primum crasso, prominenti, integro et roundato, demum rimoso-crenulato, flexuoso, partim vel fere omnino evanescente; sporae 8, decolores, oblongo-ellipsoideae, non septatae, 10–14 × 6–7.5 μ.

Thallus thick, closely adnate, light brown, whitish pruinose over small portions here and there, the central portions partly chinky to subareolate or warty, and in part lobulate, distinctly lobed toward the margins, the lobes slightly to strongly convex, transversely more or less broken-furrowed, rather short, their borders entire to irregularly and coarsely wavy-crenate, the tips sometimes tinged blackish; apothecia small to middle-sized, 0.5–2 mm. across, subsessile, the disk flat to slightly convex, pale yellowish green pruinose, the exciple colored like the thallus, at first thick, raised, entire and round, becoming cracked-crenulate, flexuous, and partly or nearly disappearing; hypothecium and hymenium hyaline; paraphyses stout, several-septate, rarely branched toward the enlarged but scarcely colored apices; asci broadly clavate; spores 8, hyaline, oblong-ellipsoid, non-septate, 10–14 × 6–7.5 μ.

The algal host is *Protococcoid*.

On northward facing tuff at 2450 feet, near Tucson, Arizona, collected by J. C. Blumer, April 1908 (type), Fink Herb. No. *5955*.

Similar to *L. muralis* (Schreb.) Rabenh., but differing in the more irregular and whitish pruinose thallus and the pale green pruinose apothecia.

13. **Lecanora pallida prolifera** Fink, subsp. nov.

Apothecia mediocria, demum proliferata.

Apothecia middle-sized, becoming proliferate.

On trees on Mt. Pinnacle, South Carolina, collected by H. A. Green, July 12, 1886 (type), Fink Herb. No. *613*.

14. **Lecanora Sambuci minnesotensis** Fink, subsp. nov.

Excipulum demum flexuosum et evanescens; disco tum valde convexo et rupto in 2–8 areas convexas, velut ex totidem minutissimis apotheciis conglomerato.

Exciple becoming flexuous and disappearing, the disk in this condition strongly convex and broken into 2–8 convex areas, giving the appearance of as many, very minute conglomerate apothecia.

On balsam trunks about Grand Portage, Minnesota, collected by Bruce Fink, June 19, 1897, No. *25* (type), Fink Herb. No. *2318*, and again on August 12, 1902, Fink Herb. No. *5201*.

15. **Lecanora iowensis** Fink, sp. nov.

Thallus tenuis, glaucus vel canescens, subtiliter albido-pulverulentus, rimosus vel areolatus, areolis parvis, planis, rare lobatis; apothecia minuta vel parva, 0.25–0.7 mm. lata, immersa vel adnata, 1–2 in areola, disco leviter concavo vel plano, pallide vel obscure fusco vel nigricanti, velo canescenti-pruinoso et persistenti, excipulo integro, demum subflexuoso, concolore thallo vel obscuriore; sporae 8, decolores, oblongo-ellipsoidese, non septatae, $10\text{–}14 \times 5\text{–}8\,\mu$.

Thallus thin, greenish gray to ashy, finely whitish pulverulent, chinky to areolate, the areoles small and flat, rarely lobed toward the margins; apothecia minute to small, 0.25–0.7 mm. across, immersed to adnate, 1–2 in each areole, the disk slightly concave to flat, light to darker brown or blackish, beneath a persistent grayish white pruinose cover, the exciple entire, becoming somewhat flexuous, colored like the thallus or darkening; hypothecium and hymenium hyaline; paraphyses distinct, stout, enlarged and rarely branched toward the apices; asci clavate; spores 8, hyaline, oblong-ellipsoid, non-septate, $10\text{–}14 \times 5\text{–}8\,\mu$.

The algal host is *Protococcoid.*

On calcareous rocks near Fayette, Iowa, collected by Bruce Fink, April 1894 (type), Fink Herb. No. *2305*.

This plant was distributed by Prof. Fink under the name of *L. calcarea* (L.) Sommerf. A plant collected by Hall in Kansas and named *L. calcarea* was examined by Prof. Fink from the Tuckerman Collection of the Farlow Herbarium at Harvard University, and was found to be the same. Similar to *L. calcarea* but differing in the finely pulverulent thallus, the somewhat smaller apothecia and the smaller spores consistently 8 to the ascus. Also similar to *L. dispersa* (Pers.) Rolh., but differing in the more persistent thallus and the pruinose apothecia.

16. **Parmelia Finkii** Zahlbr. sp. nov.

Thallus parvus, adnatus, glaucus vel canus, subtus niger et confragosus, ramulis parvis et coralloideis, lobis demum elongatis et ramosis, apicibus integris aut rare crenatis; rhizoideis parvis et dispersis; apothecia parva, 2–3 mm. lata, disco concavo, fusco, excipulo crenato aut obscure coralloideos ramulos ferente; sporae 8, decolores, oblongo-ellipsoideae, non septatae, 8–11 × 5–6 μ.

Thallus small, adnate, greenish gray to ashy, bearing small coralloid branchlets of the same colors, the lobes becoming moderately elongated and laterally branched, sometimes imbricated, the tips entire or rarely crenate, often narrowed; black and roughened below, with few and scattered obscure rhizoids; apothecia small, 2–3 mm. across, the disk concave, chestnut-brown, the exciple crenate or bearing obscure coralloid branchlets; hypothecium hyaline to slightly brownish; hymenium hyaline or brownish below; paraphyses rarely branched; asci clavate; spores 8, hyaline, oblong-ellipsoid, non-septate, 8–11 × 5–6 μ.

The algal host is *Protococcus*.

On trees and mosses over trees, Williamsville, Wayne County, Missouri, collected by Colton Russell, March 1898 (type), Fink Herb. No. *8943*. Named and described by Dr. A. Zahlbruckner in a letter but unpublished as far as known.

Similar to *P. carolinana* Nyl. and *P. latissima* (Mont.) Fee, but differing in the thallus which is smaller than either, and having smaller spores.

17. **Caloplaca oxfordensis** Fink, sp. nov.

Thallus tenuis vel crassus, formatus e granulis minutis, planis vel convexis, sordide canescenis, dispersis vel coalescentibus in continuam areolatum crustam; apothecia minuta vel parva, 0.1–0.4 mm. lata, adnata vel subsessilia,

disco leviter concavo vel plano vel convexo, luteo vel fusco, excipulo tenui; sporae 8, decolores, ellipsoideae vel oblongo-ellipsoideae, 1-septate, loculis polaribus, 13–16 × 5.5–8 μ.

Thallus thin to moderately thick, composed of minute, flat to convex, dirty gray to darkening granules, scattered or crowded into a continuous, areolate crust; apothecia minute to small, 0.1–0.4 mm. across, adnate to subsessile, often crowded and irregular, the disk slightly concave to flat or somewhat convex, orange to brown or dusky, the thalloid exciple rather thin, orange to darker, becoming flexuous; hypothecium and hymenium hyaline; paraphyses septate, unbranched and free, more or less enlarged at the apices; asci clavate; spores 8, hyaline, ellipsoid to oblong-ellipsoid, 1-septate, the cells polar, 13–16 × 5.5–8 μ.

The algal host is *Protococcus*.

On exposed rocks near Oxford, Ohio, collected by Bruce Fink, August 9, 1909 (type).

Similar to *C. citrina* (Hoffm.) T. Fries and to *C. sideritis* (Tuck.) Fink, but differing from the latter by the somewhat thinner thallus and the smaller apothecia, from the former in the color of the thallus which is also more scattered in this species.

18. **Blastenia novomexicana** Fink, sp. nov.

Thallus formatus e squamis minutis vel parvis, convexis, granulosis viridiflaventibus vel luteis, coalescentibus in plus minusve continuam crustam; apothecia parva, 0.3–0.6 mm. lata, subsessilia, disco plano vel leviter convexo, luteo, excipulo proprio tenui, pallido aut rare concolori disco; sporae 8, decolores, oblongo-ellipsoideae, demum 1-septate, loculis polaribus 12–16 × 6–7.5 μ.

Thallus composed of minute to small, convex, greenish yellow to orange, granulose squamules, running together into a more or less continuous crust; apothecia small, 0.3–0.6 mm. across, subsessile, the disk flat to slightly convex, orange, the proper exciple thin, lighter or more rarely colored like the disk; hypothecium and hymenium hyaline; paraphyses unbranched, becoming enlarged and slightly colored at the apices, rarely and indistinctly septate; asci clavate; spores 8, hyaline, oblong-ellipsoid, becoming 1-septate, the cells polar, 12–16 × 6–7.5 μ.

The algal host is *Protococcus*.

On granite rocks near Las Vegas, New Mexico, collected by Brother Anect, November 12, 1925 (type), Fink Herb. No. *15,480*.

Similar to *B. lobulata* (Floerke) Fink and *B. modesta* (Zahlbr.) Fink, but without the marginal lobing of the thallus as found in these, and with longer spores.

19. **Rinodina kentuckyensis** Fink, sp. nov.

Thallus tenuis, levis vel minute granulosus, continuus vel dispersus, sordide canescens vel obscure niger; apothecia minuta, 0.1–0.25 mm. lata, numerosa, rotundata vel subdifformia, partim immersa vel adnata, disco concavo vel fere plano, obscure nigro vel canescenti-pruinoso, excipulo crassiusculo, integro, prominenti, concolori thallo; sporae 8, fuscae, oblongo-ellipsoideae, 1-septatae, plerumque constrictae ad septum, 15–18 × 7.5–8.5 μ, inconditae.

Thallus thin, smooth to minutely granulose, continuous or scattered, dirty gray to dull black; apothecia minute, 0.1–0.25 mm. across, numerous, round to somewhat irregular when crowded, partly immersed to adnate, the disk concave to almost flat, dull black or slightly grayish pruinose, the exciple rather thick, entire, prominent, colored like the thallus; hypothecium and hymenium hyaline; paraphyses slender, hyaline, enlarged and usually brownish toward the apices; asci clavate to broadly clavate, the wall conspicuously thickened in the apical region; spores 8, brown, oblong-ellipsoid, 1-septate, usually constricted, 15–18 × 7.5–8.5 μ, irregularly arranged.

The algal host is *Protococcus*.

On sandstone rocks in Kentucky, collected by Bruce Fink, September 4, 1912 (type).

Similar to *R. nigra* Fink, but the thallus sometimes becoming darker, the apothecia slightly smaller and the spores longer.

20. **Rinodina microbola** Tuck. sp. nov.

Thallus tenuis, crasse areolatus, canus vel candidus, areolis dispersis aut contiguis; apothecia minuta vel parva, 0.15–0.4 mm. lata, partim immersa vel adnata, disco plano vel convexo, nigro, excipulo tenui; sporae 8, fuscae, ovoideo-ellipsoideae, 3 transverse septatae et raro 1 longitudinale septatae, 13–22 × 7–11 μ.

Thallus thin, coarsely areolate, ashy to whitish, the areoles scattered or continuous; apothecia minute to small, 0.15–0.4 mm. across, partly immersed to adnate, the disk flat to convex, black, the exciple thin, colored like the thallus; hypothecium yellowish to brownish; hymenium hyaline to brownish above; paraphyses semidistinct, appearing to be somewhat branched; asci inflated-clavate; spores 8, brown, ovoid-ellipsoid, 3-septate transversely and becoming 1-septate longitudinally, 13–22 × 7–11 μ.

The algal host is *Protococcus.*

On rocks in California, the specimen bearing no collector's name or date of collection, Fink Herb. No. *11,233* (type). Also Tuckerman is given as the author but no published description has been found.

Similar to *R. conradi* Koerb., but differing in the areolate thallus and the spores which here show a submuriform condition.

21. **Rinodina bolodes** Tuck. sp. nov.

Thaluus crassus, granulosus, granulis parvis, flaventibus vel canescentibus; apothecia parva vel mediocria, 0.5–1.2 mm. lata, sessilia, disco plano vel convexo, nigro vel canescenti-pruinoso, excipulo crasso; sporae 8, fuscae, ellipsoideae vel ovoideo-ellipsoideae, 1-septatae, 14–20 × 6–8.5 μ.

Thallus thick, composed of small, coarse, yellowish to gray, convex, crowded granules; apothecia small to middle-sized, 0.5–1.2 mm. across, sessile, the disk flat to convex, black or grayish pruinose, the exciple thick, colored like the thallus, becoming flexuose; hypothecium yellowish; hymenium hyaline to brownish above; paraphyses slender, unbranched, becoming semidistinct; asci clavate; spores 8, brown, ellipsoid to ovoid-ellipsoid, 1-septate, 14–20 × 6–8.5 μ.

The algal host is *Protococcus.*

On soil, near San Diego, California, collected by C. R. Orcutt, Fink Herb. No. *11,228* (type). The specimen bears a name with Tuckerman as the author but no published description has been found.

Similar to *R. turfacea* (Wahl.) T. Fries, but differing in the much smaller spores.

22. **Rinodina ochrocea** Willey, sp. nov.

Thallus tenuis, rimoso-areolatus, areolis minutis vel parvis, inaequalibus, continuis, flaventi-canescentibus vel fuscis; apothecia minuta vel parva, 0.2–0.4 mm. lata, immersa vel adnata, disco concavo, nigro, excipulo crassiusculo; sporae 8, fuscae, oblongo-ellipsoideae, 1-septatae, 20–25 × 9–11 μ.

Thallus thin, chinky-areolate, the areoles minute to small, irregular, continuous, yellowish gray to brownish; apothecia minute to small, 0.2–0.4 mm. across, immersed to adnate, more or less crowded, round to irregular, the disk concave, black, the exciple rather thick, colored like the thallus, more or less uneven; hypothecium and hymenium hyaline; paraphyses slender, branched; asci

clavate; spores 8, brown, oblong-ellipsoid, 1-septate, 20–25 × 9–11 μ.

The algal host is *Protococcus*.

On rocks, Chester County, South Carolina, collected by H. A. Green. The specimen bears no date of collection. It is marked "from the type." Willey is given as the author, but no published description has been found.

Similar to *R. sophodes* (Ach.) Koerb., but differing in the lighter-colored thallus and the longer spores.

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DASYSCYPHAE ON CONIFERS IN NORTH AMERICA. II. D. ELLISIANA

Glenn Gardner Hahn and Theodore T. Ayers

(with plates 21–23)

INTRODUCTION

When the European larch canker disease, caused by *Dasyscypha Willkommii* (Hart.) Rehm was discovered in this country, considerable apprehension was felt concerning the introduced fungus, because of reports from Europe attributing the organism to be a parasite of Douglas fir [*Pseudotsuga taxifolia* (LaM.) Brit.]. This apprehension was increased when cankered Douglas firs associated with a related *Dasyscypha* species were discovered in New England (1928) by Howard (3) shortly after the European larch canker disease was found in the United States. In certain instances these diseased Douglas firs were growing in close proximity to the imported European larch infected with *D. Willkommii.* Accordingly it became necessary to investigate the pathology of the new Douglas fir disease, as well as the taxonomy of the *Dasyscypha* forms commonly occurring on this economically important conifer, in order to determine their relationship to *D. Willkommii.*

In a previous study, described in the first paper of a series dealing with North America coniferous *Dasyscyphae* (2), investigation has shown that the European larch canker parasite does not attack Douglas fir, but the closely related saprophyte, *D. calycina* Fuckel (nec *Peziza calycina* Schum.), may fruit sparingly on dead, or weakened and dying tissue of that host. The present paper, which is second in the series mentioned, gives the results of a related research in which *D. Ellisiana* (Rehm) Sacc., the fungus commonly fruiting in association with the Douglas fir disease, is shown to be a native species. This fungus, which has generally been regarded as a harmless saprophyte on pine, has become parasitic upon introduced species of pine and Douglas fir in New England. The taxonomic detail given, indicates the relationships of *D. Ellisiana.* Data, which have shown the organism to

be a parasite when artificially inoculated into healthy Douglas fir, will be presented in a separate paper dealing with the pathological aspects of the problem.

HISTORY AND DESCRIPTION OF DASYSCYPHA ELLISIANA (REHM) SACC.

Dasyscypha Ellisiana was described in 1876 by Rehm (6) as *Peziza Ellisiana* n. sp., from material collected by Ellis (1875) on the bark of *Pinus rigida* Mill in New Jersey. It was subsequently transferred to the genus *Dasyscypha* in 1889 by Saccardo with the following synonyms: *P. Ellisiana* Rehm; *P. calycina* Schwz. in Herb. Soc. Nat. Phila., sec. Ellis.

Rehm had distributed type specimens of the new American organism as Ascomyceten n. *303* (7th fasciculus, published 1876). Later, however, he changed his mind about his fungus, and in making the combination *Dasyscypha lachnoderma* (Berk.) Rehm, for Berkeley's new species, *P. lachnoderma* (1860) from Tasmania (**1**), he considered the American plant as a synonym of the fungus collected in the southern hemisphere. Rehm probably came to this decision because of statements made by Cooke, who suggested that the two names were synonymous for the same fungus, soon after Rehm described *P. Ellisiana*. Cooke figured (Grevillea **4**: 171–2, *pl. 66, fig. i,* 1876) a specimen collected in South Carolina and identified as *P. calycina* by Curtis. From Cooke's illustration and accompanying notes, it is apparent that Curtis' fungus was nearly related to *D. Ellisiana,* and certainly not *Dasyscypha calycina* Fuckel. (**2**). It was Cooke's opinion, however, that Curtis' fungus seemed to be *P. lachnoderma* although he did not commit himself, for the reason that he had not seen Rehm's new species, *P. Ellisiana*. In September of the same year, Cooke (Grevillea **5**: 37, 1876) stated that Phillips had communicated to him that the spores of *P. Ellisiana* agreed perfectly with those of *P. lachnoderma* Berk. Rehm apparently was governed by these opinions for when he came to distribute his " Schedula rectificata," he changed the label for " Ascom. *303 Dasyscypha Ellisiana* Rehm, in litt. ad. cl. Cooke, on *Pinus* Rinde, Newfield, N. J., N. Amerika 12/1875, J. B. Ellis,"—to "*Dasyscypha lachnoderma* (Berk. sub *Peziza*)" and referred to Cooke's observations.

Saccardo perpetuated the error when he gave Rehm's rectified specimen, Ascom. n. *303 Peziza lachnoderma,* as the exsiccatum for *Dasyscypha lachnoderma* (**7**, p. 433). At the same time Saccardo (**7**, p. 459) made the new combination *D. Ellisiana,* based upon Rehm's *P. Ellisiana,* citing the original description but not mentioning the type, Ascom. n. *303*.

Berkeley's *P. lachnoderma* (1860) was redescribed by Massee (**5**) with the synonym *Dasyscypha lachnoderma* (in part) according to Rehm, because of the latter's change of opinion with regard to *Peziza Ellisiana.* Massee, however, recognized Ellis' New Jersey fungus as a species quite distinct from Berkeley's species from Tasmania.

Since there has been so much confusion in the past regarding the identity of *Dasyscypha Ellisiana* and since certain American mycologists continue to use the name of the Tasmanian fungus for the native plant, it is important because of the pathological significance of the organism, to give at this time a complete description and indicate wherein it differs from the foreign fungus:

DASYSCYPHA ELLISIANA (Rehm) Sacc., desc. emend.

Syn.: *Peziza Ellisiana* Rehm, in Grevillea **4**: 169. 1876.

P. calycina Fries (Herb. Soc. Nat. Phila.).

Dasyscypha lachnoderma (Berk.) Rehm, pro parte, Ascom. *303;* Sacc. Syll. **8**: 433, 1889.

D. Ellisii Rehm.

Helotium Ellisianum Wettstein.

Apothecia commonly gregarious (PLATE 21, FIGS. 1, 2; PLATE 23, FIGS. 3, 4), or scattered, shortly but distinctly stipitate; stem white (not black at base); at first pyriform, margin incurved and closed, then expanding when fully mature becoming nearly plane, with corrugated margin more or less upraised (PLATE 21, FIG. 1), silky margin incurved when dry, fringed with fasciculate hairs; externally pure white or yellow to yellowish-green, densely downy; hairs elongate, flexuous, filamentous, septate, hyaline, smooth, with short cells, sometimes swollen or bulbous, 10–18 × 2–5 μ, thin-walled, cylindrical, obtuse, persistent, (PLATE 22, FIG. 1); disc light orange-yellow to deep chrome,[1] 0.5–2 mm. diam., commonly 1 mm. diam.

[1] The color nomenclature used is that of R. Ridgway, Color standards and color nomenclature, 1912, Wash., D. C.

Asci clavate, short-stalked, obtuse, (100) 54–72 × 5.5–7.0 μ (PLATE 22, FIG. 3). Ascospores 8, irregularly biseriate, hyaline, smooth, continuous at first, unicellular or uniseptate upon germination, (PLATE 21, FIGS. 3, 4), straight, fusiform, ends acuminate, (100) 16.0–27.9 × 1.4–2.9 μ, commonly, 17–21 × 2–2.5 (PLATE 22, FIG. 5). Paraphyses filamentous, straight or slightly flexuous (see Cooke, Grevillea 4: *pl. 66, fig. i*), septate, obtuse or acuminate at tips, (100) 72–90 × 1–2 μ (PLATE 22, FIG. 3) occasionally somewhat broader above with the tip narrowed.

Imperfect stage abundant, yellowish-green, minute, consisting of an erumpent stroma, 106–132 μ diam., at first closed, then opening up with a single exposed chamber (PLATE 23, FIG. 1) or compound, with more than one locule, 243–433 μ diam. (PLATE 23, FIG. 2). Microconidia fusiform, 5.0–5.8 × 0.9–1.2 μ (PLATE 22, FIG. 7) abstricted from the tips of short sporophores, subulate, acute, simple or verticillately branched (PLATE 22, FIG. 6).

The imperfect stage, which is here reported for the first time, was found commonly in New England during the summer, followed by the perfect stage which formed during the autumn and winter. However, ascocarps containing spores could be collected throughout the entire year. With age the hymenium was observed to disintegrate, or was eaten out by insects leaving behind the excipular shells of the fruit cups. These persisted (PLATE 23, FIG. 4) and could be recognized readily as bleached, white remnants tinged with deep glaucous-green or light porcelain-green, with which the new immature ascocarps were associated.

The hosts upon which *D. Ellisiana* has been found and their distribution according to states are grouped together in the following list:

On Abieteae. *Larix europaea* D. C.,—N. Y., R. I.; *Larix leptolepis* Murr.,—Mass.; *Picea Engelmanni* Engelm.,—Mass.; *P. pungens* Engelm.,—Mass.; *Pinus austriaca* Asch. & Graebn.,—Conn., N. J.; *P. Banksiana* Lamb.,—Conn., Mass., R. I.; *P. Cembra* L.,—Conn.; *P. echinata* Mill.,—N. Car.; *P. flexilis* James,—Mass.; *P. monticola* D. Don.,—Mass.; *P. nigra Poiretiana* Asch. and Graebn.,—Ohio; *P. ponderosa* Lawson,—Mass.; *P. pungens* Lamb.,—N. Car.; *P. resinosa* Sol.,—Conn., Maine, R. I.; *P. rigida* Mill.,—Conn., Maine, Mass., N. J., N. Car., Pa., W. Va.;

P. Strobus L.,—Maine, Mass., N. J., N. C., Pa., R. I.; *P. sylvestris* L.,—Mass., N. J., Pa., R. I; *P. taeda* L.,—Del., La., N. J.; *P. virginiana* Mill.,—Md., Pa., Va.; *Pinus spp.*,—Ala., Miss., Fla., S. Car., Texas; *Pseudotsuga taxifolia* (LaM) Brit., blue form,—Mass., N. Car., R. I.

Exsiccata examined:

Besides the numerous collections filed in the collections of the Division of Forest Pathology at New Haven, Conn., the writers have examined the following herbarium specimens collected on various hosts in North America and found them to be *Dasyscypha Ellisiana*. In many instances these specimens previously had been identified as *D. lachnoderma.*

Peziza calycina Fries, Syn. Car. *1207, vulgaris* Bethlehem *55,* on pine bark, Schweinitz Herb. *1831* (Phila. Acad. Nat. Sci.).

Peziza calycina, on bark of pine, Houston, Texas, *171,* H. W. Ravenel, 1869 (Herb. Myc. B.P.I., U.S.D.A.). According to an annotation on the specimen packet, Dr. W. W. Diehl had identified this specimen as *D. Ellisiana.* We did not examine the poor material microscopically but macroscopically the fungus appears to be that species.

Peziza lachnoderma Berk. on pine branches, Aiken, S. C., *175* Ravenel, Fungi Am. (N. Y. Bot. Gard.).

Dasyscypha lachnoderma (Berk. sub. *Peziza*), Schedula rectificata, Ascom. n. *303, Dasyscypha Ellisiana* Rehm, on pine bark, Newfield, N. J., coll. J. B. Ellis, Dec. 1875 (N. Y. Bot. Gard.).

Peziza Ellisiana Rehm, nov. spec. on *Pinus rigida,* Newfield, N. J., Dec. 1875, n. *716,* Thümen Myc. Univ. (N. Y. Bot. Gard.).

Peziza lachnoderma Berk., on pine bark, Newfield, N. J., July, 1882 in Ellis collection (Herb. N. Y. Bot. Gard.). A specimen *P. Ellisiana* Rehm, coll. Ellis, pine limbs, Newfield, N. J., Jan. 1876, in Remainder Herb. Ellis, purchased C. L. Shear,—bears the following interesting annotation by Ellis who had identified the fungus originally as *P. calycina* Schum.:—" Mr. Peck thinks this is near *Peziza Ellisiana.* Disc pale straw-yellow."

Peziza lachnoderma Berk., = *D. Ellisii* Rehm. = *D. lachnoderma* Rehm on *Pinus* dead branches, Ocean Springs, Miss., Feb. 1, 1887, (Herb. F. S. Earle, N. Y. Bot. Gard.).

Dasyscypha lachnoderma Berk. on *Pinus rigida,* Arlington Hts., Mass., May 21, 1894, (Burt Herb. in Farlow Herb., Harvard Univ.). *D. Ellisiana* was found in this packet although Burt included illustrations of a *Dasyscypha* with oblong spores, 14–17 × 5–6 μ, with the specimen.

Peziza (Dasyscypha) lachnoderma Berk., bark of dead *Pinus austriaca* (cult.) Newfield, N. J., Dec. 1894. Ellis & Ev. N. Am. Fungi, *3231.* Also, *D. lachnoderma,* N. Am. Fungi, *3231,* Dec., 1894 (N. Y. Bot. Gard.).

Dasyscypha lachnoderma (Berk.) Rehm on *Pinus,* dead bark, Auburn, Lee Co., Ala., July, 1896. Coll. F. S. Earle (N. Y. Bot. Gard.).

Dasyscypha lachnoderma (Berk.) Rehm on pine bark, Auburn, Lee Co., Ala., March 21, 1897. Coll. F. S. Earle, *2236,* Ala. Biolog. Survey (N. Y. Bot. Gard.).

COMPARISON WITH DASYSCYPHA LACHNODERMA (BERK.) REHM

As previously indicated Massee (5) had pointed out correctly that *D. Ellisiana* was distinct from *D. lachnoderma.* He emphasized certain important differences: ". . . Dr. Rehm described . . . under the name of *Peziza Ellisiana,* a minute *Peziza* growing on pine bark collected by J. B. Ellis at Newfield, N. J., U. S. A. This species was afterwards issued in Rehm's Ascom. n. *303* as *Dasyscypha Ellisiana* Rehm. Some time afterwards Rehm sent out a new label for his Ascom. n. *303,* in which he substituted the name *Dasyscypha lachnoderma* (Berk.) Rehm. This was unfortunate, as the American plant is quite distinct, differing from *P. lachnoderma* Berk., more especially in the following points: Ascophore smaller, not so distinctly stipitate; stem not black; externally tinged yellowish-green; asci slightly broader, spores shorter and thicker, and in all the specimens I have examined continuous; always growing on bark or wood of conifers. Hence *Peziza Ellisiana* Rehm, must stand as a species distinct from *P. lachnoderma* Berk., with the following synonymy: *Dasyscypha lachnoderma,* Rehm, Ascom. n. *303;* Sacc. Syll. VIII, n. 1804 (in part). . . ." The authors have examined the exsiccata listed by Massee, which he regarded as being *Dasyscypha Ellisiana,* and have confirmed him in his opinion.

In his redescription of Berkeley's *Peziza lachnoderma,* Massee (5) reported the fruit bodies of the Tasmanian fungus as being larger than *Dasyscypha Ellisiana,* 3–4 mm. in diameter, and having a stipe which is conspicuously black at the base. This is illustrated by Massee on his original plate of *D. lachnoderma* preserved in the herbarium of The New York Botanical Garden. His notes and drawings made from the type specimen collected by Archer on dead bark in Tasmania (1) and deposited at Kew, not only clearly illustrate this particular character, but also give other morphological detail in agreement with his description.

The writers have examined a specimen of *Dasyscypha lachnoderma,* collected by Rodway in Tasmania, from the herbarium of Massee (Herb. N. Y. Bot. Gard.). Their observations on this fungus have confirmed those of the English mycologist. The following morphological characters indicate outstanding dissimilarities with *D. Ellisiana.* The excipular hairs are elongate, flexuous, thin-walled, minutely roughened, with long cylindrical cells (16.0–41.2 $\times$ 3.0–4.2 μ) and obtuse extremities (PLATE 22, FIG. 8). In the examination of herbarium material, the character of the hairs was found to be most useful in distinguishing the two species when spores were lacking. The asci (PLATE 22, FIG. 9) are cylindrical, clavate, apex slightly narrowed, long-stalked (20) 68.4–93.6 $\times$ 4.0–4.8 μ (Massee gave somewhat shorter measurements,—60–70 $\times$ 6–8 μ). The ascospores (PLATE 22, FIG. 10) are irregularly biseriate, hyaline, smooth, crescent-shaped, with acuminate apices, one to three septate, (50) 12.6–24.4 $\times$ 1.4–2.4 μ (according to Massee, 23–30 $\times$ 2 μ). The paraphyses, which Massee (5) described as "very slender, very slightly thickened at the tip, and the point narrowed, delicately septate," were present. These paraphyses, which measured 97–112 $\times$ 2–4 μ (PLATE 22, FIG. 9), could be considered lance-like in that they possessed "apice acutae" and were "grandiusculae" in the sense of Karsten's *Lachnum* (4).

For the reason that Rehm recognized the genus *Lachnum,* one would naturally expect him to have called the *Peziza lachnoderma* of Berkeley, *Lachnum lachnoderma* (Berk.), instead of *Dasyscypha lachnoderma* (Berk.), when he (Rehm) made that new combination. Rehm probably did not actually examine Berkeley's material and merely based his opinion upon the published statements

of Cooke and Phillips, that his new species, *P. Ellisiana,* agreed with the Tasmanian plant. At the time he made the combination, Rehm no doubt had in mind *P. Ellisiana,* which can be considered a species of *Dasyscypha;* for as Rehm (6) had described, it possessed paraphyses which were "filiformes, ascos superantes, septatae, c. 2 μ crass." Because of the broad, acerose paraphyses of *P. lachnoderma,* this species should be transferred to the genus *Lachnum* as **L. lachnoderma** (Berk.) comb. nov.

Inasmuch as *D. Ellisiana* has slender, filamentous, straight or slightly flexuous paraphyses, which possess extremities either obtusely rounded or acuminate, the authors have preferred to keep the species within the genus *Dasyscypha* (2). The paraphyses of this species resemble those illustrated for *D. cerina* (Pers.) Fuckel, in Clements and Shear (The Genera of Fungi, *pl. 33,* 1931). *D. Ellisiana* would appear to be a borderline species between those forms having filamentous paraphyses and those producing broad, lanciform structures.

LIFE-HISTORY STUDIES

Since cultures from fresh material of *Lachnum lachnoderma* were not available for study, observations on those made with *D. Ellisiana* can only be reported at this time. Cultures of the American fungus were obtained from diseased tissues of the host (PLATE 23, FIG. 3) and from mono-ascus and -ascospore isolations. The spores germinated readily on 3 per cent malt agar under ordinary laboratory conditions within 48 hours, producing polar germ tubes at one or both ends. The ascospores as in the case of the large-spored, white-excipled *Dasyscyphae* (2) became commonly uniseptate or germinated without producing a septum (PLATE 21, FIGS. 3, 4). Cultures were also obtained from single paraphyses (PLATE 22, FIG. 4) or excipular hairs (PLATE 22, FIG. 2) which were found to be capable of continued growth, producing characteristics similar to those obtained in cultures from spore isolations. Cultures obtained in this way gave rise to the formation of the imperfect stage.

On malt-agar, single-spore cultures of *D. Ellisiana* produced a fine, silky, snow-white, aerial mycelium, which in the early stages of the culture had the appearance of "combed wool" (PLATE 21, FIG. 6). It was noted that certain cultures became somewhat appressed and as staling set in, ceased to produce outwardly a vig-

orous vegetative growth (PLATE 21, FIG. 5). In the former type of colony a beautiful sulphur yellow color appeared which changed with age to a light porcelain-green, pale glaucous-green or dusky green-blue. In the latter type of colony, pinkish-buff or cinnamon-buff appeared becoming interspersed with the green shades mentioned. The agar under the mycelial mat was discolored, so that it became tawny, seal brown or plumbeous brown. A physiological difference has been noted among strains of *D. Ellisiana*.

It was discovered that *D. Ellisiana* is able to produce in culture a soluble, non-volatile, thermostable, crystalloidal substance, which formed readily in the presence of certain sugars or fresh Douglas fir extract, but not in the presence of proteins. This non-living constituent which was toxic to Douglas fir and other conifers, causing browning of needles and defoliation, apparently does not inhibit the growth of the organism; for fresh malt agar tubes, whose slant surfaces had been moistened with the toxic substance, when inoculated with the fungus, produced normal growth.

The imperfect stage formed readily from single ascospore cultures. The fruit bodies, however, showed a tendency to become compound under artificial conditions, forming extensive stromatic growth, oozing glaucous-green or dusky green-blue masses of the microconidia or spermatia. The sporophores and conidia produced in culture are of the same type as those formed in nature. Numerous attempts to germinate these conidia have failed. Attempts have also been made to spermatize fresh single ascospore isolation cultures with these microconidia, to induce the formation of the perfect stage but without success. We believe the spermatia germinable although we have not been able to demonstrate this. It is interesting here to note that we failed to obtain germination of the microconidia of *D. Ellisiana* and the four species of the large-spored *Dasyscyphae* (2). However, we did succeed in obtaining the germination of the microconidia of species of the *D. calyciformis* group. The small, unicellular ascospores of species belonging to this last-named group, germinated readily without forming a septum, whereas the ascospores of species producing microconidia which did not germinate, formed one or more septa during the germination period.

The imperfect stage of *D. Ellisiana* was found abundantly occurring during the summer. By late autumn the perfect stage,

which persisted into the late spring, had formed among these conidial stromata upon the bark scales of pine, or upon the resinous lesions and bark of the diseased Douglas fir. In southern New England the ascospores were fully mature in May and June.

DISCUSSION

A perusal of the exsiccata examined and reported in this paper will show that while *Dasyscypha Ellisiana* has been widely collected on pine in the eastern United States, it has generally been identified as *D. lachnoderma*. Despite the fact that it has recently been observed occurring commonly on the blue Douglas fir in New England, particularly where this western species is growing in a badly diseased condition (3), the occurrence of Ellis' and Rehm's fungus upon this host has heretofore been unrecognized. It is of interest to remark here that Seymour (Host Index of the Fungi of North America, 1929), does not cite *D. Ellisiana* on Douglas fir in his compilation of published fungus records, and where the organism is mentioned, it is reported only on one host. On *Pinus rigida* both *D. lachnoderma* and *D. Ellisiana* are listed; on *P. nigra* and an undetermined species of *Abies,* the former fungus.

As far as we have been able to determine *Lachnum lachnoderma* has been collected only in the southern hemisphere and in Cuba. As previously stated the type came from Tasmania. According to Massee (5), " other specimens in the herbarium are from Brisbane (Bailey, nn. *572* and *1804*) : Natal (MacOwan, nn. *156, 194, 1126*). The type of *P. melanopus,* Berk. & Curt., from Cuba (Wright, n. *368*), proves to be identical with *P. lachnoderma.* Not any of the specimens mentioned above are growing on bark or wood of conifers."

Apparently in the case of *D. Ellisiana,* we have an instance of a native saprophytic fungus which has become parasitic on introduced Douglas fir, western yellow and limber pines and Swiss stone pine (*Pinus Cembra*) in New England. The species has been known in the literature since the days of Schweinitz (1831) who probably first collected it (8). Our pathological research, which has consisted of a very large number of artificial inoculations upon Douglas fir, western yellow pine and eastern and western white pines, has shown the fungus to be parasitic. Cankers have been obtained experimentally, upon which the imperfect and per-

fect stages of the species fruited on the dead cortex. In our inoculation work, strains of *D. Ellisiana* obtained from diseased bark tissue and from mono-ascus and -ascospore isolations were used. As has been pointed out in a previous paper (2), *D. Willkommii,* the European larch canker parasite, unlike *D. Ellisiana,* does not infect either healthy or weakened Douglas fir.

A complete search for the fungus has not been made. However, from miscellaneous collections we know it to occur from Maine to Texas in close proximity to the coast line; for to date it has been collected in only one instance in a locality more than 400 miles inland. Hedgcock collected it on Mt. Pisgah, N. C., which has an elevation of 5,749 feet. *D. Ellisiana* has been taken only once very recently in the midwestern states (*53885, Pinus nigra Poiretiana* Shawnee State Forest, Ohio, coll. R. K. Beattie and Curtis May, May 1, 1933) and not in the far West. The late Ellsworth Bethel and Drs. Seaver and Shope, who have collected Discomycetes widely in Colorado, never came upon it. Mr. J. R. Hansbrough of the Division of Forest Pathology, who collected *Dasyscyphae* for these investigations (2) and who knew *D. Ellisiana* intimately, did not find it either on pine or Douglas fir on the western coast or in the Pacific Northwest (including British Columbia).

D. Ellisiana is not reported from Europe. The senior author in company with Mr. Ivar Jørstad (Skogdirektorens innberetning om det Norske Skogvesen for 1930, p. 88, Oslo, 1931) has observed blue Douglas fir seriously diseased in Norway (Søfteland) in 1927–1928, but the *Dasyscypha* abundantly concerned in this particular instance as a probable parasite, was another species, *D. resinaria* (Cooke & Phill.) Rehm.

SUMMARY

A taxonomic study of *Dasyscypha Ellisiana* (Rehm) Sacc., commonly associated with diseased Douglas fir (*Pseudotsuga taxifolia,* blue form), growing within and outside areas in New England where the European larch canker fungus, *D. Willkommii* (Hart.) Rehm, was found introduced, has shown the organism to be a native species.

D. Ellisiana, which was first collected in 1831 by Schweinitz as *Peziza calycina* Fries, has been generally regarded as a saprophyte

on pine. Recent observations have shown that the organism apparently has become parasitic on four introduced host species in New England—*Pseudotsuga taxifolia* (blue form), *Pinus ponderosa, P. flexilis* and *P. Cembra.*

D. Ellisiana has been frequently confused taxonomically with *D. lachnoderma* (Berk.) Rehm, a non-coniferous Discomycete from Tasmania. These two species, as Massee pointed out, are quite distinct. Morphological data confirming Massee's observations are given setting forth the differences between them together with notes upon life-history studies of *D. Ellisiana.*

An amended description of *D. Ellisiana* is included in which the imperfect stage is reported for the first time. The species is reported on 15 species of pine, on spruce, on larch, and on blue Douglas fir. *D. Ellisiana* occurs along the seaboard from Maine to Texas. It has been collected only once in the Mid-West and not in the far West or in Europe.

D. lachnoderma is now considered as belonging to the genus *Lachnum* and is called *Lachnum lachnoderma* (Berk.) Hahn & Ayers. According to Massee, the fungus occurs in the southern hemisphere and has never been found on the bark or wood of conifers. Cuban material, identified as *Peziza melanopus* Berk. & Curt., is regarded by Massee as identical with the Tasmanian fungus.

We wish at this time to express our appreciation to the officials of Farlow Herbarium, Harvard University, to the Director and Dr. F. J. Seaver of The New York Botanical Garden and to the officials of the Herbarium, Division of Mycology, Bureau of Plant Industry, Washington, D. C., for courtesies and privileges they have accorded us. Also due recognition should be given to Mr. J. R. Hansbrough, Division of Forest Pathology for all the earlier culture isolation studies. To the late Dr. N. O. Howard and to the other members of the same Division who have sent us specimens, we are also grateful.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
IN COÖPERATION WITH THE
OSBORN BOTANICAL LABORATORY,
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EXPLANATION OF PLATE 21

Dasyscypha Ellisiana (Rehm) Sacc.

Fig. 1, Habit, fully mature apothecial material from Potowomut, Rhode Island, collected and photographed by the late Dr. N. O. Howard, March 30, 1928. On bark of cankered *Pseudotsuga taxifolia,* blue form. Approx. × 4; 2, Habit apothecia on hypertrophied bark of diseased Douglas fir material, Potowomut, R. I. Note: clumped growth of over-mature fruiting cups, slowly degenerating, leaving the bleached, whitish excipular shells. Approx. × 4; 3, Germinating ascus, after five days on the surface of malt agar. Approx. × 670; 4, Ascospores showing characteristic polar germ tubes. Approx. × 300; 5, Forty-day-old cultures on malt agar; culture on right from a mono-ascus, in the middle from mono-ascospore (North Beverley, Mass., diseased Douglas fir material *43567*), on the left from mono-ascospore (material from same locality, *43566*). Cultures show staling. Nat. size; 6, Three, 40-day-old mono-ascospore cultures on malt agar (Beverley, Mass. material, *43566*). Nat. size.

EXPLANATION OF PLATE 22

Dasyscypha Ellisiana

All drawings made with camera lucida × 800

Fig. 1, Excipular hairs; 2, Excipular hairs showing vegetative growth. *Pinus resinosa* material on malt agar; 3, Asci and paraphyses; 4, Paraphyses showing vegetative growth. *P. resinosa* material on malt agar; 5, Ascospores; 6, Sporophores, fresh *P. resinosa* material, Potowomut, R. I.; 7, Microconidia (spermatia), culture material from mono-ascospore *53169* Douglas fir, Potowomut.

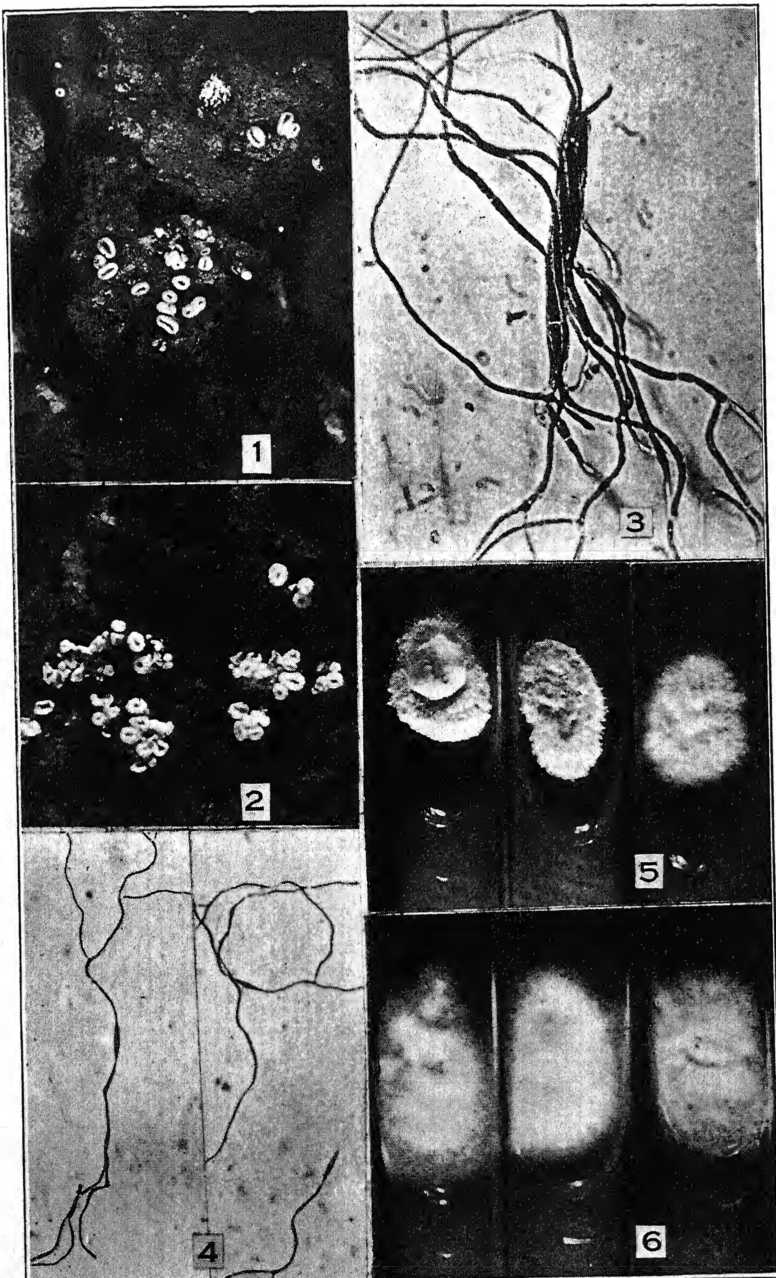
Lachnum lachnoderma (Berk.) Hahn & Ayers

Fig. 8, Excipular hairs; 9, Asci and paraphyses; 10, Ascospores.

EXPLANATION OF PLATE 23

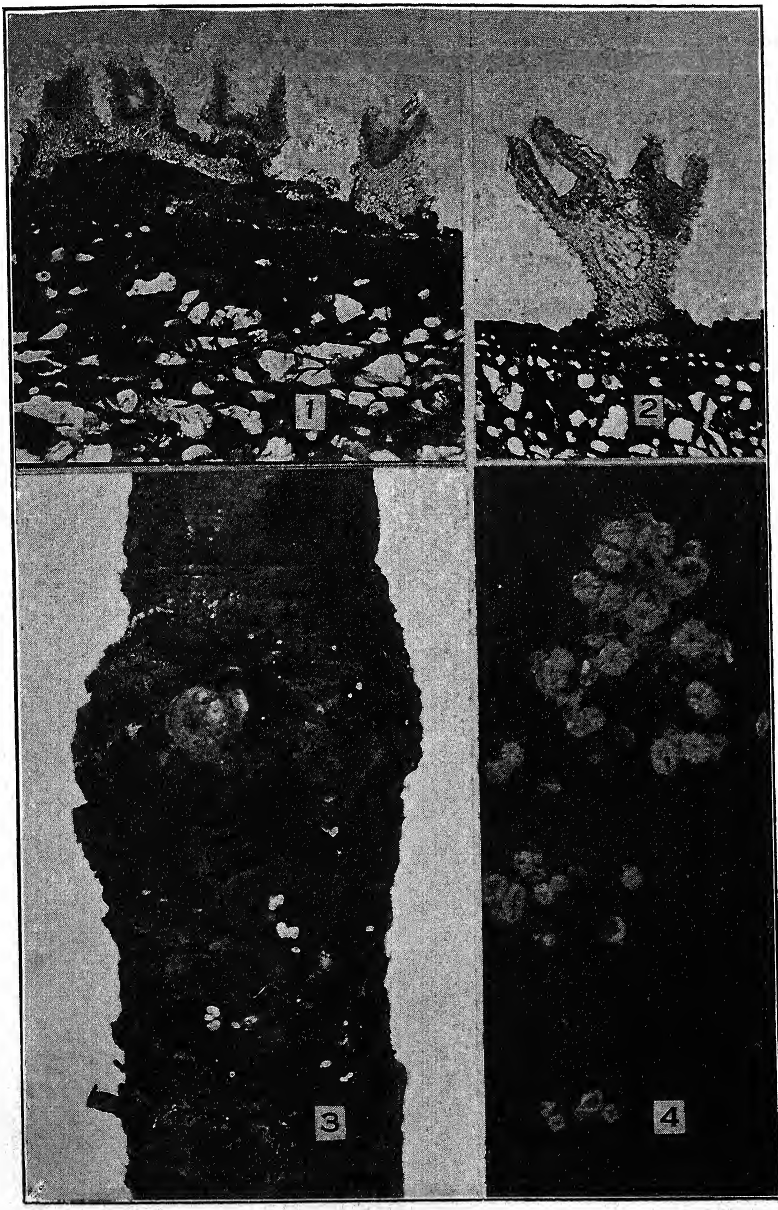
Dasyscypha Ellisiana

Fig. 1, Longisection of stromata of the imperfect stage showing simple cavities widely distended. × 65; 2, Longisection of compound stroma of the imperfect stage. × 65; 3, Diseased branch Douglas fir, Potowomut, R. I. material, showing cankered swollen base with fruit bodies on surface of hypertrophied bark. A virulent strain of the fungus, which produced the toxin peculiar to the fungus, was isolated from tissues of this canker. Approx. × 3; 4, Habit, over-mature apothecia showing disintegrating cups, the hymenia of which are disappearing (Potowomut, R. I. Douglas fir material, *43587*). Approx. × 8.5.



Dasyscypha Ellisiana

1–7. Dasyscypha Ellisiana
8–10. Lachnum lachnoderma



Dasyscypha Ellisiana

GYMNOSPORANGIUM MYRICATUM IN RELATION TO HOST PARENCHYMA STRANDS [1]

B. O. Dodge

(with plates 24 and 25 and 2 text figures)

Some of the effects of the bayberry rust, *Gymnosporangium myricatum*, on the southern white cedar, *Chamaecyparis thyoides*, were noted in a recent number of the Journal of The New York Botanical Garden [2] where photographs of two trees that had died prematurely from severe infections were shown. Such illustrations are proof that species of this rust genus can, on occasion, be very destructive to the cedar hosts. When one follows the path taken by the fungus in each case as it invades the cedar host he understands why the abnormal growths produced are sufficiently characteristic to be specifically diagnostic in most cases.

Some years ago Wörnle [3] made a study of several species of *Gymnosporangium* from the standpoint of the forester. He was particularly interested in finding out what host tissues were invaded by the parasite. One of the species studied he referred to as *G. juniperinum* on the branches and trunk. This comes the nearest to what I find for *G. myricatum* in the way it attacks its cedar host, and the histological picture he draws of the effects of *G. juniperinum* represents fairly well what one would find in examining *Chamaecyparis* infected with our species. Wörnle also made a superficial study of *G. myricatum* (*G. Ellisii*) from a single small branch, but he could not have hoped to learn many of the details from this small amount of dried material. He noted certain brown patches in the wood and cortex which contained hyphae. The hyphae, he said, were very coarse, having a diameter

[1] Studies in the genus *Gymnosporangium*—IX.

[2] Jour. N. Y. Bot. Gard. **35**: 41–45. 1934.

[3] Wörnle, P. Anatomische Untersuchung der durch Gymnosporangium-Arten hervorgerufenen Missbildungen. Forst. Nat. Zeits. **3**: 68–84; 129–172. 1894.

of 8 μ. Hyphae of the European species he found were very much finer.

As noted in an earlier publication,[4] a few telial sori develop on leaves the first spring after inoculation, but it commonly requires about 20 months. Sori first appear directly on leaf blades or on the youngest branches that are not yet covered with cork layers. The mycelium is found throughout the spongy parenchyma of the leaf beneath the sorus, and in the cortex of the young branch. Just as in all other species studied, the terminal cells of the young

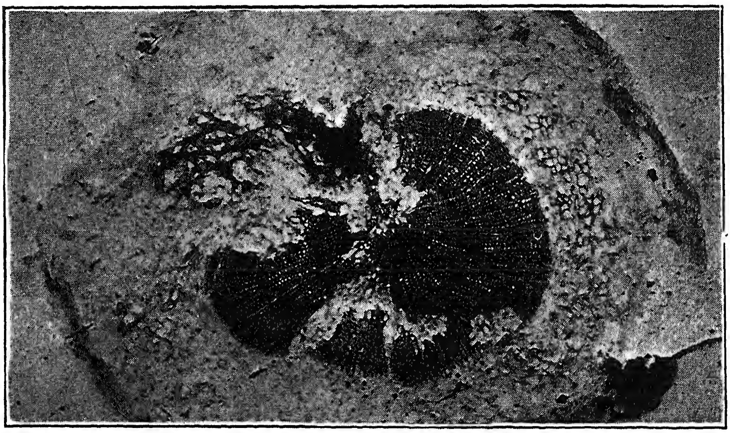


Fig. 1. Section of young *Chamaecyparis* stem infected with *Gymnosporangium myricatum.* The gaps in the wood ring occupied by thin-walled parenchyma cells accompanied by hyphal strands. Infection originally at about this point or at the time when the tip was at this point.

sorus primordium swell and elongate to make a buffer tissue to break up the overlying epidermis. Teliospores arise from subterminal cells. The necessity for such a buffer tissue is more apparent when sori in later years develop on branches or on the main trunk and are, therefore, overlaid with tough bark.

Whenever the sori break out on the trunk of a tree near the base, one may be unable to account for the appearance of the fungus on such old parts. Wörnle, in referring to the peculiar distribution of mycelia of *G. juniperinum,* suggested that the

[4] Studies in the genus *Gymnosporangium*—II. Report of cultures made in 1915 and 1916. Bull. Torrey Club 45: 287–300. 1918.

fungus originally entered the branch or trunk through the dormant buds, "schlafende augen" he called them, or through shoots because they seem to afford the only line of communication between the outside and the inner tissues now showing mycelial hyphae. This may be true in some cases, but it is more than likely that whenever the main trunk shows infection in later years the fungus originally gained entrance at the time when this part was the tip end of the main axis. If the mycelium ran down from an infected branch at that point, one should find remains of the branch still containing hyphae. Study of many southern white cedars infected with *G. myricatum* shows that the green tip of the main axis may become infected directly through the leaves at a time when this part is not covered with cork layers. In sections of a small infected stem, for example (PLATE 24, *A*) one sees gaps in the ring wood now occupied by thin-walled parenchyma cells containing haustoria. Hyphae penetrate to the very center of the twig as shown in text figure 1. As the twig grows the fungus tends to progress vertically and radially in fascicles, leaving portions of the cortex parenchyma entirely free (PLATE 25, *D*). There are many cells still capable of division distributed here and there all through the bark parenchyma. Such cells can become functional cambium and thus give rise to tracheids, which in turn cut off or surround the parenchyma strands carrying the fungus. The small picture (PLATE 24, *A*) shows, upper left, a cross section of two vertically growing parenchyma strands about to be walled in by new wood. At the right in the same figure there is shown such a strand only recently completely walled in.

Sections of a young tree show the phloem region in which the cells are more or less radially arranged, and stereome or lignified cells disposed in open rings. Such lignified cells are also distributed sparsely and rather irregularly throughout the cortex or bark parenchyma. The cells of the medullary rays, which soon collapse in the wood rings, are very conspicuous in the cortex, retaining their cytoplasmic and nuclear contents. While in cross sections one sees a great many cells of the cortex which appear to be more or less lifeless because they are somewhat collapsed and irregular, nevertheless many of them still contain nuclei and some cytoplasm and are thus capable of being rejuvenated.

Whenever a hypha advances in the vicinity of any of these medullary ray cells or any other cortex cells still possessing nuclei, such cells are rejuvenated. They become more turgid and swollen, and the nuclei are more conspicuous. After the nuclei divide, thin walls cut across the cell as though by internal division, the old

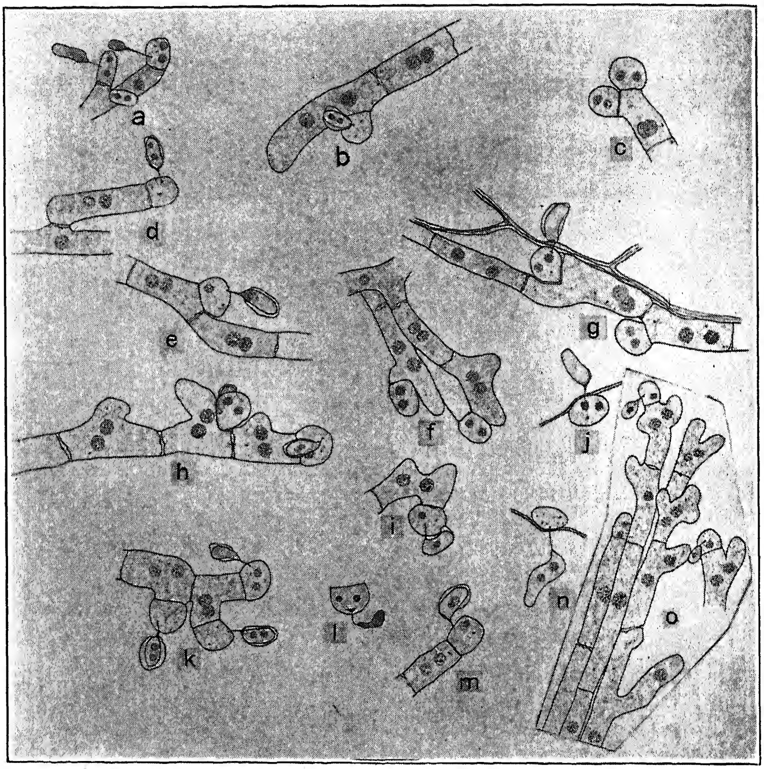


Fig. 2. Various stages in the formation of haustoria and the migration of the nuclei from the mother-cell to the haustorium. The host parenchyma cells invaded are not shown. See text for further discussion.

wall remaining intact. When a number of parenchyma cells are thus stimulated to increase in size and to divide, pressure is exerted on the neighboring cells which are forced aside or pressed together (PLATE 24, *C, D*). This gives us the picture of a parenchyma strand burrowing into the cortex, as it were, like a cancerous growth. Furthermore, one finds cases where stimulated cortex

cells multiply and add entirely new cells at the terminus of the parenchyma strand, thus simulating even more closely an invading growth.

Haustoria penetrate cells without seeming to have any deleterious effect. When a red cedar becomes infected by *G. germinale,* cells bordering the hyphae, especially cells invaded by haustoria, soon show much disorganization. *G. myricatum* has quite the opposite effect, since cell life is prolonged and rejuvenated. As the branch increases in length the fungus also grows upward as well as downward, always keeping the hyphae grouped in synemata so that sections show the parenchyma strands in different aspects depending on where the sections are made. The condition illustrated in PLATE 25, *C* would suggest that the strands had pressed the tracheids aside with great force. The true explanation is that the strands were there before the wood was laid down. Pressure was exerted on all sides of the cells about to become lignified and this kept them oriented in the way we now find them. The cells of the parenchyma strands elongate in the direction taken by the bordering hyphae and maintain life for a long time even after being surrounded by wood tissue. Cells of hyphae imbedded in three or four wood rings may still show their two nuclei plainly. Usually, however, the cytoplasm and nuclei of infected cells degenerate after the second year and the parenchyma strands turn brown (PLATE 25, *B*). Hyphal growth must be slow. Tip ends of radially growing hyphae are very conspicuous as they emerge from the wood ring and appear to be growing into the cortex (PLATE 24, *A*). No matter what the section picture of strands isolated in the wood cylinder may be, any strand, if followed up in serial sections, must invariably lead to the cortex, because the hyphae originally came in through the cortex or from the growing region in case of tip end infections before differentiation of the wood cylinder. Wherever a hypha grows, sending haustoria into the adjacent cells, it prevents those cells from differentiating sufficiently to become normal tracheids. So we have these parenchyma strands always accompanied by hyphae. The stimulus may extend to cells in the vicinity which are not touched or bordered by hyphae.

As noted previously, the hyphae of this species are comparatively coarse and have a peculiar way of branching (TEXT FIG. 2). Each cell contains two large nuclei. No other species studied has such large haustorium mother-cells. They show fairly well even in a photograph (PLATE 24, *F*). They are usually formed terminally and the subterminal cell branches out at an abrupt angle to continue onward growth. This suggests the method by which conidia of *Phytophthora* are formed. They may appear to be lateral, but they were formed terminally. The mother-cells always contain two small nuclei at first (TEXT FIG. 2).

An infected plant that had been kept in the cold frame until December 1 was brought into the greenhouse. Sections of the stem at the point of infection made two weeks later showed haustoria in all stages of development as though the fungus had just begun to invade these regions. Young haustoria were more numerous in the cortex, but they were also present in parenchyma strand cells well imbedded in wood rings. Very likely these began their development a year or two previously, and not just recently, but the nuclei were unable to pass out from the mother-cell. Perhaps a haustorium can function very well for a time without a nucleus. Dead haustoria such as shown in text figure 2, *l*, are not common. Here the haustorium is shrunken, and one sees plainly a thickened pad on the wall of the mother-cell at the point where the thread is attached. Stages like that shown in text figure 2, *j*, where the nucleole seems to have been left behind while the nucleus is spinning out to pass through the haustorium thread, are rather common. A later stage is shown at *e*. The nucleus has just reached the haustorium and stains very deeply, the nuclear membrane not being at all conspicuous. Many haustoria that appear to be fully mature show only one nucleus (TEXT FIG. 2, *m*). In this case the other nucleus is always found back in the mother-cell. When a haustorium shows two nuclei one often finds two small deeply staining specks in the mother-cell (TEXT FIG. 2, *k*), with an open space around each. If this were the size of the nucleus one might think that it represents the region originally occupied by the mother-cell nucleus and the deeply staining speck would represent the nucleole. The vacuole is much larger than the nucleus, however, and the specks are not always very definite.

There is no conspicuous swelling of the host cell wall at the point of penetration (TEXT FIG. 2, *g*). The haustorial thread is very fine, scarcely visible at the point where it passes through the host cell wall. It gradually widens and swells out at the outer end, as shown in text figure 2, *a*. As the haustorium elongates it increases in size toward the mother-cell. There is no ring or cup at the base of the haustorium such as one sees so conspicuously in *Diplocarpon Rosae*.[5]

Just what may be the nature of the so-called haustorium sheath is a question here as elsewhere. Young haustoria without nuclei seldom show any sheath, and some old haustoria do not. Usually, however, one can make out very distinctly a hyaline region bordered by a very distinct line surrounding the haustorium. This is usually more or less irregular. It seems to be attached to the haustorial thread at its outer extremity or the point where it connects with the body part.

The size of the haustorium of *G. myricatum* varies somewhat, but it is no larger when found in the cortex of a tree trunk than when it occurs in the spongy parenchyma of the leaf. The large haustorium (TEXT FIG. 2, *n*) was from a leaf cell. This point is important because there is a question whether one of the leaf infecting forms of *Gymnosporangium* on *Chamaecyparis*, the form I have previously but erroneously referred to as *G. fraternum*, may not be merely a leaf form of *G. Botryapites*. Only infection experiments would settle this question. I was never able to infect *Chamaecyparis* with either the leaf form or the stem form, using spores from aecia on *Amelanchier* leaves, aecia which would pass as belonging to *Roestelia Botryapites*. These aecia were obtained in the one case by sowing spores from telia of the leaf form, the one erroneously referred to as *G. fraternum*, and in the other case by sowing spores from telia of the stem form *G. Botryapites*. None of the sowings of the aeciospores on the *Chamaecyparis* gave telia, either of leaf form or the stem form. This is not strange because very few persons have ever been able to infect the cedars with *Gymnosporangium* species. In spite of this lack of proof, however, by studying the haustoria of the leaf infecting form and com-

[5] Dodge, B. O. A further study of the morphology and life history of the rose black spot fungus. Mycologia **23**: 446–462. 1931.

paring these with haustoria found in the cortex of the stem infecting form, one sees that there is considerable difference, although in shape and number in a cell they are very much alike. In both forms it is not uncommon to find as many as six or eight haustoria in one cell. In both forms they are rather blunt and chubby. They differ, however, considerably in size, those of the stem form being much larger. Whether the change in tissues attacked would be followed by corresponding changes in the size of the haustoria is very doubtful. The haustoria of *G. Juniperi-virginianae,* where they are found in very large gall cells, are themselves very small, in fact the smallest haustoria I have found in the genus *Gymnosporangium,* and, as noted above, the haustoria of *G. myricatum* when found in the leaf cells are just as large as those found in the cells of the branches or trunks.

G. juniperinum and *G. myricatum* are much alike, as noted previously, in the way their mycelia are distributed among the host tissues. According to Wörnle's cross section diagram of the former species, the parenchyma strands run out radially, while they may run in any direction in case of the latter species. It has been found that the histological picture of the host parasite relation and the morphology of the haustorium furnish together a valuable addition to the diagnostic features which may be useful in distinguishing species of the genus *Gymnosporangium.*

SUMMARY

The mycelium of *Gymnosporangium myricatum* penetrates the cortex parenchyma of *Chamaecyparis* near the growing region and runs along vertically or radially between the host cells in synemeta. Each hyphal cell contains two large nuclei. Binucleate haustorium mother-cells are usually formed terminally, the subterminal cell branching out to continue the growth.

A thin haustorial thread penetrates the wall of the host cell continuing on into the cytoplasm for some distance before swelling up to form the haustorium. This grows to nearly its full size without a nucleus. The nuclei from the mother-cell spin out through the thread, one following the other after a rather long interval. While many haustoria function for a time without any

nucleus, eventually a mature haustorium contains two nuclei and the haustorium mother-cell none.

Parenchyma or medullary ray cells adjacent to hyphae are stimulated to enlarge and divide. From this there results a sort of parenchyma strand. Four or five daughter cells are often enclosed within the old wall of the original cell, the daughter cells separated merely by thin membranes. New cells may be added on at the end of a strand. The pressure exerted by this forward growth of the strand often crushes the opposing cortex cells, so that there is a certain amount of invasion of cortex by a "growth." In general, however, the strand is mainly made up of cells that have been rejuvenated by the presence of the fungus. Sections of branches killed by the fungus show the remains of the strands as brown patches or streaks.

Young seedlings or branches infected in the growing region are apt to be permanently dwarfed and die early. Large trees bearing many witches' brooms may die prematurely.

The parenchyma strands never *invade* the wood rings. If found there, it means that they were captured by the wood laid down around them. The reaction of the host to the stimulus coming from these invading hyphae, and the morphology of the haustoria and their mother-cells are rather characteristic for each species of the genus and thus furnish additional clues for identification purposes.

THE NEW YORK BOTANICAL GARDEN

EXPLANATION OF PLATES

Gymnosporangium myricatum on *Chamaecyparis*

PLATE 24

A. Cross section showing a fan-shaped parenchyma strand with accompanying hyphae. The size of the hyphae is apparent from the way they show in the photograph which is not highly enlarged. At the inner end or vertex of the strand and connected with it there is seen an irregular patch of parenchyma tissue. Here the strand extends vertically so that the cut ends of hyphae as well as of the long parenchyma cells give the spot a different aspect. Where a strand is growing vertically in the phloem region it will eventually be captured and surrounded by tracheids, as shown by the whitish patches at the border of the wood cylinder.

B. Section of a dwarfed tree about 17 years old. The first ten rings are normal and show no patches with hyphae. These appear in the eleventh

ring in this section. Some distortion of the twelfth to fourteenth rings is evident. Farther up or down some section might have shown the fungus at the very center (see text fig. 1). The long radial streak represents a parenchyma strand with accompanying hyphae.

C. Parenchyma strand from the wood cylinder invading the phloem and cortex parenchyma.

D. More highly magnified picture of the outer portion showing thin-walled parenchyma cells of the strand containing conspicuous nuclei.

E. A still more highly magnified section of the outer end of a similar strand showing hyphae bordering large parenchyma cells. Note the thin walls laid across these large cells cutting them up into segments, each with its nucleus. The original wall encloses the three or four daughter cells. The crushing effect on the cortex parenchyma by the invading strand is evident here.

F. Portion of a radial strand under oil immersion lens at the border of the wood cylinder. Haustorium mother-cells with their two nuclei are conspicuous. Abnormal tracheids, lower right. Note also how the parenchyma cells elongate in the direction taken by the adjacent hyphae.

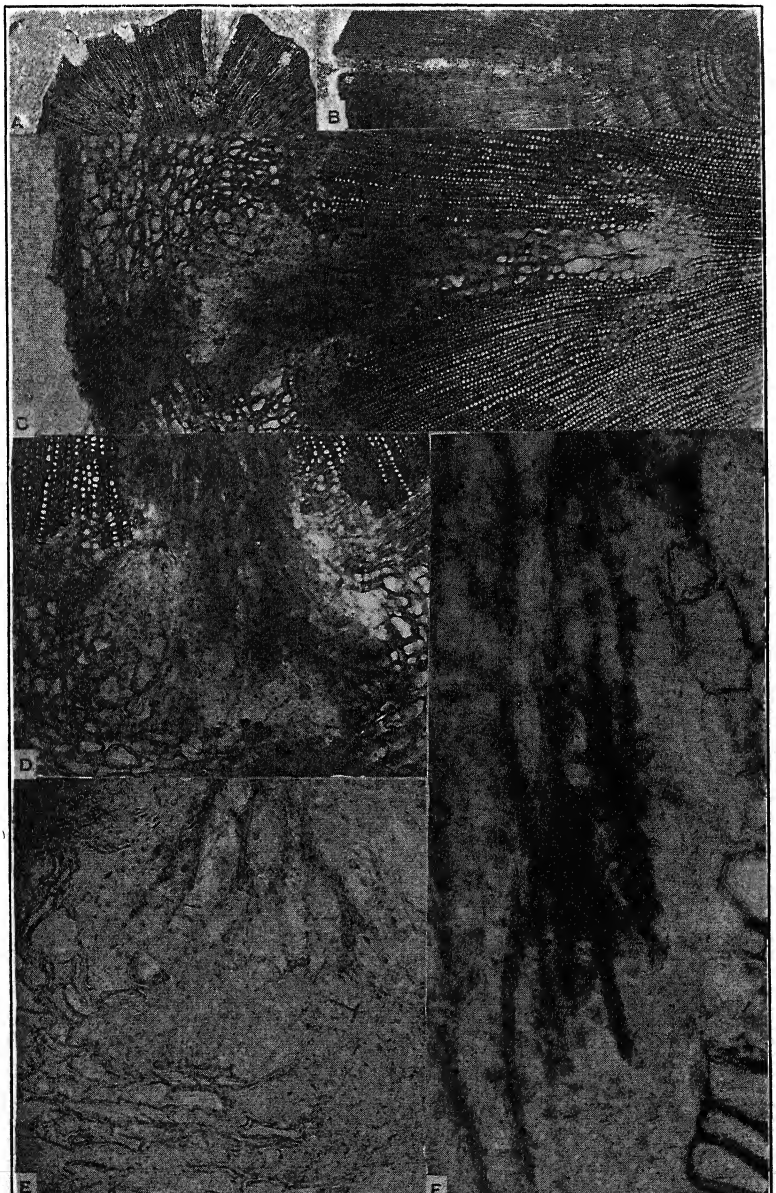
PLATE 25

A. Longitudinal radial section showing an oblique section of a parenchyma strand. Note that the wood cells have abnormal ends where they touch the strand.

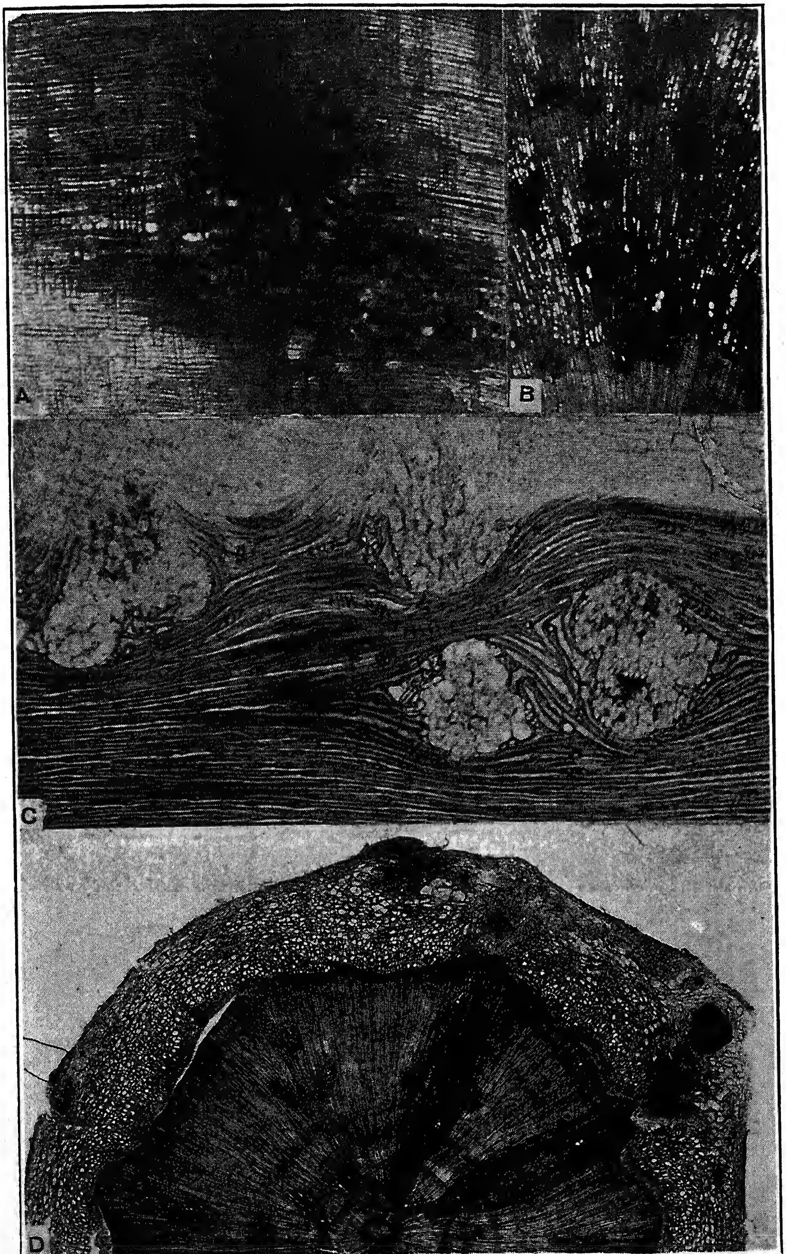
B. Cross section showing brown pockets in wood rings occupied by the fungus.

C. Tangential section showing cut ends of parenchyma strands in the wood rings, also parts of radially growing strands in the cortex. These strands were present before the tracheids, which now appear to be under great pressure. They were simply forced to take up their present positions as they were built up around the strands.

D. Cross section showing two radially growing parenchyma strands. Several vertically growing cortex strands are cut across. The black spot in the cortex at right center represents one of Wörnle's "schlafende augen" or dormant buds. No hyphae were found associated with this one.



Gymnosporangium myricatum



GYMNOSPORANGIUM MYRICATUM

NOTES AND BRIEF ARTICLES

Mycologia Endowment Fund

On May 11, 1933 the Managing Editor received a check for one thousand dollars ($1000.00) from one who wishes to be referred to merely as "a friend of MYCOLOGIA." At the suggestion of the Managing Editor this was accepted by the Board of Managers of The New York Botanical Garden and set apart as the nucleus of an endowment fund with the following resolution:

> "RESOLVED, that the gift of $1,000 from an anonymous donor be accepted, subject to the provisions of confidential letter on file with the Secretary, and the Director is hereby instructed to express the very sincere thanks of the Executive Committee to the very generous contributor.
>
> RESOLVED FURTHER, that the gift of $1,000, referred to in the above minute, be added to the restricted endowment funds, the income only to be used for the support of MYCOLOGIA."

It is expected that this fund will be added to from time to time either by private donation or funds from the sale of back sets published previous to 1933. Added contributions are solicited.

PHYTOPATHOLOGICAL CLASSICS

Under the above title the American Phytopathological Society is publishing a series of papers, three of which have already been issued as follows:

1. Fabricius. Attempt at a dissertation on the diseases of plants. 1774. Translated from the Danish by Mrs. Margaret Kølpin Ravn.
2. Fontana. Observations on the rust of grain. 1767. Translated from the Italian by P. P. Pirone.
3. Millardet. The discovery of Bordeaux mixture. Three papers. 1885. Translated from the French by F. J. Schneiderhan.

These may be had at fifty cents each or for a limited period at one dollar and twenty-five cents for the three. Application should be made to H. H. Whetzel, Cornell University, Ithaca, New York. Other numbers are in course of preparation.

A Note on Mycology in Brazil Today

Stationed at the Agricultural College of the State of Minas Gerais, Brazil, during the last four years, the writer of this note had occasion to meet various Brazilian mycologists, and had access to numerous agricultural and scientific publications of Brazil today.

Perhaps the most prolific author during that time has been Padre Joáo Rick, of Porto Alegre, Rio Grande do Sul, who has published in almost every number of the journal *Egatea,* volumes 14–18, on the Basidiomycetes, particularly the Hymenomycetes. There have appeared, under his authorship, lists, including diagnoses of many new species of fungi collected in that state belonging to the following families: Thelephoraceae, Hydnaceae, Lycoperdaceae, Nidulariaceae, Phallaceae, and Hymenogastraceae. Monographic studies have appeared on *Agaricus, Boletus,* and *Helvella,* by the same author in the Portuguese journal, *Broteria.*

Three mycologists, collectors over a period of some thirty years in eastern central Brazil, and possessors of excellent private herbaria are Drs. Eugenio Rangel, Arsene Puttemans, and Rosario Averna-Sacca, and they are still active. Padre C. Torrend, collector of Basidiomycetes, is Brazil's northernmost mycologist.

Drs. Heitor Silvevio Grillo and Ageslau Bitancourt are two young Brazilian scientists, who, trained in mycology, are transferring their efforts at present more to the study and control of plant diseases and to the development of this science in Federal and State Agricultural Departments. These workers are greatly desirous of contacts and of exchanging material and information with colleagues in other lands, as is the writer who continues as mycologist and plant pathologist at the College at Vicosa, Minas Gerais, Brazil.

ALBERT S. MÜLLER

Pilobolus crystallinus in Pure Culture

Ordinarily *Pilobolus crystallinus* (Wiggers) Tode has been grown in the laboratory only upon its natural substratum, *i.e.* the dung of herbivorous animals. Only recently an artificial medium has been used for its cultivation. On April 14, 1933 [1] the writer

[1] Annual meeting of The Arkansas Academy of Science.

presented an account of his culture of this fungus on a dung agar. This agar was prepared as follows:

"Boil 300 gms. fresh sheep dung in a liter of distilled water until the dung is broken down. Filter. Restore liquid to its original volume. Add 15 gms. agar agar shreds. When agar is melted place in 250 cc. Erlenmeyer flasks. Autoclave for 15 min. at 15 lbs. pressure. Slant flasks to obtain maximum surface of the agar."

No difficulty was encountered in securing pure cultures from single sporangia, or from sporangiophores with sporangia attached. The surface of such flasks became covered with characteristic fruiting structures which were discharged in the normal way. The glass on the side of the flask opposite the slanted surface was very closely dotted with the discharged sporangia. A culture prepared in November, 1932 which began to discharge sporangia December 1, 1932 is still discharging sporangia, but in considerably less numbers on May 8, 1933.

Pilobolus crystallinus has also been grown on a beef extract agar which may be prepared as follows: "Boil 210 gms. boiling beef vigorously in a liter of distilled water until the meat is thoroughly cooked. Strain the broth through several thicknesses of cheese cloth. Restore to original volume and make agar as described above."

Transfers made from a dung agar culture to a flask of this beef agar on April 30, 1933 produced the first crop of sporangia on May 5, 1933. The fungus is quite readily adaptable to this medium and thrives quite well. Subcultures made from such cultures continue to grow and produce normal sporangia. In older cultures many of the sporangia are not discharged with as much force as they are in younger cultures. In previously prepared cultures, now three months old, sporangia are still being produced, although they are not being discharged so violently.

From these results it is evident that:

1. It is unnecessary for spores of *Pilobolus crystallinus* to pass through the digestive tract of an animal before germinating.

2. *Pilobolus crystallinus* may be easily cultured in pure culture on an ordinary dung decoction agar without any involved treatment.

3. *Pilobolus crystallinus* may be grown for several successive generations on artificial media at ordinary laboratory temperatures.

4. This fungus has been grown in the laboratory on a beef decoction agar, and has thrived for some time.

DELBERT SWARTZ

UNIVERSITY OF ARKANSAS

MUSHROOM POISONING AT SEATTLE

The popular and widespread interest in mushrooms around the Puget Sound region has gradually increased from year to year until now it has become almost phenomenal. Whether this interest has been augmented by the present depression it is hard to say. From the time the rains start in the fall until the frost comes there is an ever-increasing interest. Usually the spring is less favorable for the growth of mushrooms in this region but owing to the wet and late spring this year (1933) there was a more than usual interest exhibited.

This mushroom gathering was not entirely free from unpleasant incidents. At times some over-enthusiastic or careless collectors made rather serious mistakes in their identification of edible species. Eight cases of mushroom poisoning caused from eating *Amanita pantherina* Fries have recently come under my notice. This species was first reported for the United States in 1929 by Zeller who found it rather abundantly in the spring in Oregon. He also reports several cases of mushroom poisoning as a result of eating this species. The following report emphasizes his findings.

Early in May, in the vicinity of Anacortes, Washington, two people, Mr. and Mrs. R., aged about 35, both in fine physical condition, prepared specimens of *A. pantherina* in the usual way and ate them at noon. In less than an hour both became very dizzy and felt weak and sick. A neighbor drove them immediately to a physician. By this time the woman had collapsed and the man showed inability to coördinate, acting as if drunk. The physician sent me the following statement: " The patients were immediately taken to the hospital, the woman rapidly losing consciousness and developed spasmodic jerkings of her whole body. Both had subnormal temperatures, dilated pupils and pulse as low as forty. The woman became quite violent, dizziness and inability to think

seemed to be the most pronounced symptoms. There was no pain, colic, etc. at any time. The treatment was as follows: A pint of permanganate of potash solution was immediately given, followed by one-tenth grain of apomorphine, then one-fiftieth of a grain of atropin. Following emesis and complete cleaning out of the stomach, each was given one ounce of castor oil and as soon as this worked they were given one-quarter grain of morphine and one-fiftieth grain of atropin. During all this eliminative treatment each was given a teaspoonful of a solution consisting of ten drops tincture agaricus muscaris in a half glass of water, every half hour. The man did not lose consciousness but lay in a semi-comatose condition for several hours. The woman regained consciousness in about eight hours. By the next morning both appeared normal and were sent home, and the following day they were practically recovered except that they were both very weak."

A little later in May two other persons, Mr. and Mrs. S. found the same species which had been collected by some one and left by the roadside. Believing these to be discarded mushrooms they were taken home, cooked and eaten with much the same results. The man, however, had a weak heart which could not withstand the extra work put upon it. He died. The woman survived.

About two weeks later four other Seattle residents driving out in the country one Sunday afternoon collected what they believed to be edible mushrooms but they proved to be *A. pantherina.* All four were taken violently ill with much the same symptoms as those described for the two at Anacortes. After a few days illness all four recovered.

J. W. Hotson

University of Washington,
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MYCOLOGICAL SOCIETY OF AMERICA

Report of the Highlands Foray

The Mycological Society of America, at its foray in August, 1933, voted that a list of species taken by each collector should be filed with the Highlands Museum and Biological Laboratory, and that these records would form the basis of a report, here published.

The total number of named species collected during the three days of the foray is 430, distributed in groups as follows: Agaricaceae, 156; Ascomycetes, 85; Polyporaceae, 47; Thelephoraceae, 39; Fungi Imperfecti, 24; Uredinales, 17; Gasteromycetes, 15; Hydnaceae, 15; Boletaceae, 14; all others, 18. Several other species collected await determination. Some of these, it now appears, are new to science.

A considerable proportion of the species found are of rather general occurrence and are therefore omitted from this notice. On the other hand, several collections are of interest; some have not previously been reported from North America, and others, according to published records, are rare or limited in their range.

MYXOMYCETES.—*Cribraria microcarpa* (Schrad.) Pers., *Arcyria pomiformis* (Leers) Rost., *Echinostelium minutum* deBary., *Hymenobolina parasitica* Zukal.

ASCOMYCETES.—*Thelebolus lignicola* Lloyd, *Elvela atra* Oed., *Calicium polyporaeum* Nyl, *Balansia Hypoxylon* (Peck) Atk., *Bombardia fasciculata* Fries, *Cordyceps clavulata* (Schw.) Ellis & Ev., *Hypoxylon regale* Morgan, *H. serpens* var. *macrospora* Mill., *Rosellinia Clavariae* on (Desm.) Tul. on *Clavaria cristata* (Holmsk.) Fries, *Scoleconectria scolecospora* (Bref.) Seaver, *Xylaria ianthino-velutina* Mont.

FUNGI IMPERFECTI.—*Sepedonium brunneum* Peck, *Toxosporium abietinum* Vuill., *Gonatobotryum maculicolum* (Wint.) Sacc.

UREDINALES.—*Puccinia Acetosae* Körn.

TREMELLALES.—*Tremella* (*Naematelia*) *aurantia* (Schw.) Burt.

THELEPHORACEAE.—*Aleurodiscus apiculatus* Burt, *Corticium byssinum* (Karst.) Massee, *C. confusum* Bourd. & Galz., *C.* (*Botryobasidium*) *coronatum* (Schröt.) v. Höhn. & Litsch, *C. subcoronatum* v. Höhn. & Litsch, *C. tulasnelloideum* v. Höhn. & Litsch, *Peniophora attenuata* Bourd. & Galz., *P. pallidula* Bres., *Septobasidium pinicola* Snell, *Stereum burtianum* Peck, *S. Murrayi* (Berk. & Curt.) Burt, *S. pallidum* (Schw.) Lloyd, *Thelephora albido-brunnea* Schw., *Tremellodendron cladonia* (Schw.) Burt.

HYDNACEAE.—*Odontia cristulata* Fries.

POLYPORACEAE.—*Polyporus balsameus* Peck, *P. fragilis* Fries, *P. graveolens* Schw., *P. Montagnei* Fries, *P. Pes-caprae* (Pers.)

Fries (= *P. retipes* Underw.), *P. poculus* Schw., *Solenia fasciculata* (Pers.) Fries.

BOLETACEAE.—*Boletus cyanescens* (Bull.) Fries, *B. parasiticus* (Bull.) Fries.

AGARICACEAE.—*Amanita spreta* Peck var. *parva* Beardslee, *Armillaria* (*Pleurotus*) *corticata* Fries, *Cantharellus floccosus* Schw., *C. infundibuliformis* (Scop.) Fries, *C. tubaeformis* Fries, *Clitopilus abortivus* Berk. & Curt., *Cortinarius largus* Fries, *Crepidotus stipitatus* Kauff., *Inocybe agglutinata* Peck, *I. decipientoides* Peck, *I. leptocystis* Atk., *I. prominens* Kauff., *I. umbrinella* Bres., *Lactarius atroviridis* Peck, *L. mucidus* Burl., *L. peckii* Burl., *Leptonia lampropoda* Fries, *L. subserrulata* Peck, *Panus laevis* Berk. & Curt., *Pleurotus atropellitis* Peck, *Russula aeruginea* Lindbl., *Tricholoma decorosum* Peck, *T. leucocephaloides* Peck, *T. portentosum* Fries var. *centrale* Peck, *T. subsejunctum* Peck, *T. transmutans* Peck.

L. R. HESLER

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DEPARTMENT OF BOTANY.

REPORT OF THE SECOND ANNUAL MEETING

The second annual mid-winter meeting of the Mycological Society of America was held December 28, 29, and 30, at Boston, Massachusetts, in conjunction with that of the American Association for the Advancement of Science. The Society headquarters were at the Hotel Westminster on Copley Square in Boston. The sessions were held at Harvard University in Cambridge. The meeting was well attended, and, though hindered somewhat by extremely inclement weather, will be remembered as a pleasant and profitable one. The local arrangements made for us by Doctor Weston were most satisfactory. The retiring president, C. L. Shear, presided at the sessions, and, at the close of the business meeting on Thursday, addressed the Society. His paper, entitled "Mycology, Scientific and Otherwise," will appear in the next issue of MYCOLOGIA. The Society held joint sessions with Section G and the American Phytopathological Society. Saturday afternoon was given to the making of demonstrations of research mate-

rials and to the explanation of mycological exhibits set up earlier in the week by members of the Society.

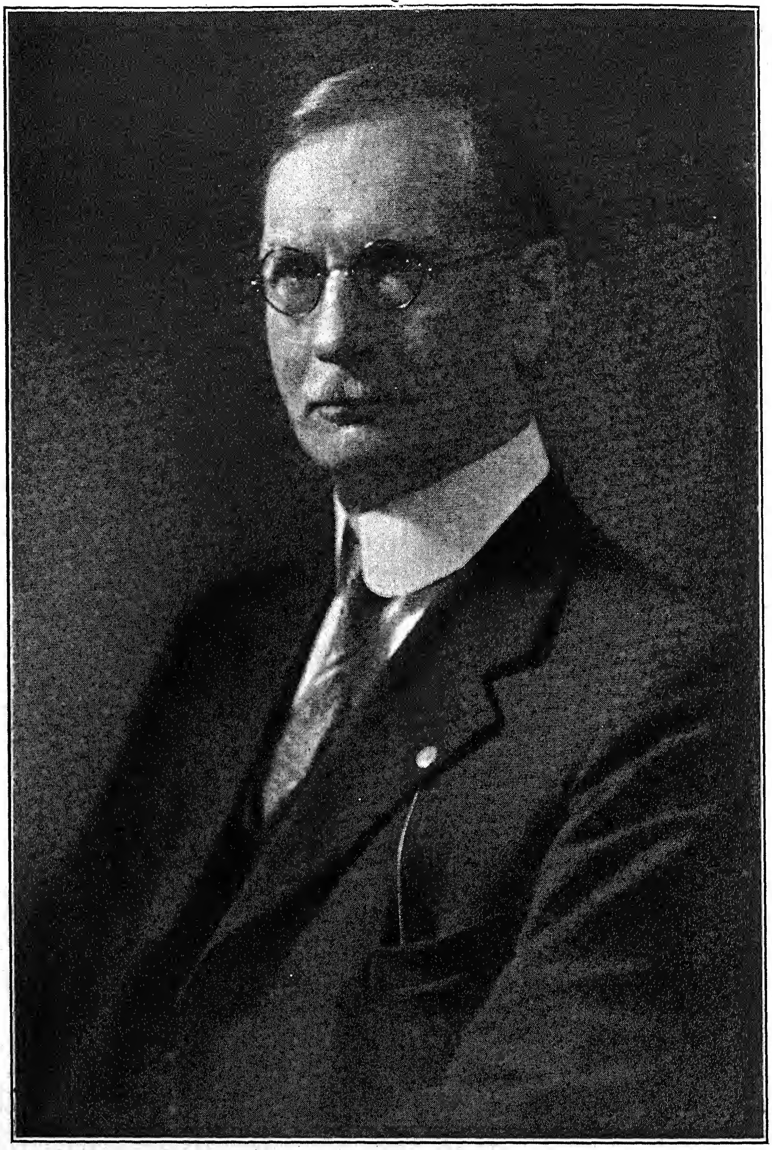
At the business session on Thursday, reports by the secretary-treasurer and managing-editor of MYCOLOGIA showed the Society and its journal to be in satisfactory financial condition. New officers elected for 1934 are Herbert S. Jackson, president; Bernard O. Dodge, vice-president; and Lee O. Overholts, councilor. The council named G. W. Martin to succeed H. M. Fitzpatrick on the editorial board of MYCOLOGIA. The editor-in-chief, F. J. Seaver, reported that the board had voted unanimously against the printing of abstracts of papers presented at the meetings. This action was accepted as final disposition of the matter. The amendments to the constitution submitted to the membership by mail in November were adopted. One of these empowers the council to name a Society Historian. As yet the position has not been filled.

At the regular sessions approximately thirty-five papers were presented, dealing with many groups and phases of mycology. The prominence of contributions on the Phycomycetes, noted last year at Atlantic City, was again evident. An increase in interest in the field of medical mycology was indicated. Papers on aspects of sexuality in Ascomycetes and Basidiomycetes were outstanding. A paper by S. M. Zeller, not received in time for inclusion in the printed program, was read on Thursday, the title being "Protogaster, a representative of a new order of Gastromycetes." A brief discussion was given by J. C. Arthur of his Manual of Rusts, now in press. Doctor Arthur was introduced at the Botanists' Dinner as the "Dean of American Botany," and was most cordially received. He was also retiring president of the American Phytopathological Society.

The council approved the printing of an address list of our members. This will be prepared and mailed as soon as the necessary data are available. Those who have not yet returned the blank provided for this material are urged to forward it promptly. An increase in our membership is desirable. All persons interested in mycology are eligible and are invited to join. At present the roll consists of approximately three hundred and fifteen names.

A detailed statement of all receipts and expenditures accompanied by vouchers was submitted to the council. This was audited and approved. The auditing committee, named by the president, consisted of H. S. Jackson, chairman, Neil Stevens, and L. Leonian.

H. M. FITZPATRICK, *Secretary-Treasurer*



Cornelius Lott Shear

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XXVI MAY–JUNE, 1934 NO. 3

MYCOLOGY, SCIENTIFIC AND OTHERWISE [1]

C. L. SHEAR

(WITH PORTRAIT)

Since mycology is a branch of science, its study should be pursued in a strictly scientific manner, that is, we should seek the truth without prejudice so far as it is humanly possible.

QUALIFICATIONS AND PREPARATION

Let us consider for a moment what qualifications and preparation are essential for success in mycological work. As is sometimes said of teachers, it may be said of mycologists, the greatest are born rather than made; that is, they inherit that innate love of nature and absorbing interest in all of her objects and works which helps to overcome all the difficulties and discouragements encountered in the pursuit of knowledge. Assuming the possession of the natural qualifications, we may consider the preparation necessary. Unfortunately our present home and school influences in many instances tend to discourage rather than to develop the natural interests and tendencies of children toward the study of nature. One of the most serious defects of our scientific training today, is too early specialization. The student should have a broad training in general science and courses in general botany, including the taxonomy of the flowering plants. It is pathetic to find professional pathologists and mycologists who do not know

[1] Address of the retiring President of the Mycological Society of America, Boston, Mass., December 28, 1933.

[MYCOLOGIA for March–April (**26**: 113–199) was issued April 2, 1934]

the common wild plants which are the hosts of the fungi they study. I would not insist, however, that this be carried so far as to expect a mycologist to give the latest specific name to a specimen of *Crataegus* or *Rubus*.

Besides a broad view and concept of science in general, the student should have a good knowledge of languages, literature, and philosophy; as these subjects should all be useful as well as enjoyable in connection with the strictest scientific investigations and conclusions. A knowledge of the general history and development of science, and especially of botany and mycology, is of the greatest value in helping one to get a proper perspective and appreciation of the problems presented. The training must be broad and thorough in order to follow successfully what Tulasne calls "that most difficult of botanical paths, the way of mycology."

If one is preparing for taxonomic work, extensive and thorough field as well as herbarium studies must be made; and it is only after years of field, laboratory, and herbarium work with the best facilities and an abundance of material, as well as opportunity to study authentic and type material, that one can hope to successfully undertake monographic work and make a real contribution to our knowledge of the genera and species and their true relationships. It seems a mistake in most cases to encourage students to undertake monographic work for theses. One realizes this more and more as the years go by and we begin to get a real understanding of the difficulties involved.

My own experience may or may not have a bearing on this subject, depending on how it is interpreted. For nearly forty years I have been accumulating material and studying the species of *Xylaria* and *Hypoxylon,* as opportunity occurred, hoping some time to prepare a monograph of these genera. The more extensive our field studies, the greater the quantity of material examined, the more difficult it becomes to decide generic and specific limits. With little experience and scanty material, it may seem rather easy to distinguish these groups; but when you have an abundance of material from a very wide range of localities, many variations and intergrading forms are found which multiply the difficulties of segregation. Of course, this may all simply go to show that much study is not only a weariness of the flesh, but a cause of

mental confusion; or that your humble servant does not possess the divine afflatus necessary to recognize genera and species. Whatever the conclusion, the important thing is the demonstration and recording of the fact that these variations and intermediate forms do exist and must be reckoned with in our interpretation of plants and their relationships.

MOTIVES AND IDEALS

Motives and ideals are as important in scientific work as elsewhere, if one is to attain any real and permanent success. The primary motive in mycology should be the discovery of truth, advancement of our knowledge of the fungi, and the utilization of this knowledge for the physical, intellectual and social improvement of mankind. The old advice to "hitch your wagon to a star" still holds good, but, unfortunately, under the present depressing conditions the natural tendency is to hitch to anything that will bring bread and butter. Purely selfish motives, personal vanity, and desire for publicity, may appear to lead to temporary success, but do not usually secure for one a prominent or permanent place on the roll of honor of distinguished contributors to the advancement of science. It may be pointed out incidentally, that all our present social, economic, and political troubles are directly traceable to selfishness; a fact so obvious that few apparently recognize or admit it. Our ideal should be to attain as far as possible exact and complete knowledge of the organisms we study, in all their aspects and relations. The recognition of our vast ignorance should teach humility rather than pride and assurance. If one seriously contemplates the great marvels of organic life in any of their multitudinous and complex phases, it is difficult to understand how he can fail to be impressed with the magnitude of the problems which confront him and realize that, at best, we can only approximate the real truth. If we could really get an adequate conception of the difficulties to be encountered we might become totally discouraged and give up all effort.

Unfortunately, some of the work in systematic mycology at present can scarcely be called scientific. The multiplication of so-called "new species" based upon insufficient knowledge of those already described, insufficient material for study, and an

inadequate concept of species, has led to a multiplicity of synonyms and added to the confusion which already existed.

The practice of basing "new species" on difference of host, or even difference of part of host, especially of saprophytes or more or less omnivorous parasites, is certainly otherwise than scientific. If physiological differences between organisms can be demonstrated on different hosts or parts, they should be designated as physiological forms. The systematist should recognize that fungi, as most other plants, are in process of evolution and a state of unstable equilibrium; in other words, are constantly varying. The condition in one genus or species may, however, be much more unstable than that in a closely related group. What characters are most variable and to what degree and under what conditions, must be determined in each case by the most careful observation and field and laboratory study of an abundance of material from various localities.

To prevent further over-production of new species, it may be necessary to have an NRA code, strictly limiting the number anyone may produce per annum and also, perhaps, requiring a license to be issued only to those who pass a rigid examination showing that they have the necessary training, experience, and discrimination to recognize genuine species.

SOME PRESENT NEEDS

We may perhaps be permitted to point out here what we consider some of the greatest present needs for the advancement of systematic mycology. One of these needs is a thorough study and re-description of all the available type or authentic material of the species of fungi of the older authors; a very large number of which are at present imperfectly known or misinterpreted. Probably not one-fourth of the species found in Saccardo's "Sylloge Fungorum" are well enough described or known to be identified with certainty. Consequently, many names are differently applied by different mycologists, which causes much misunderstanding and confusion. Much valuable work in this direction has been recently done by von Höhnel and others, some of which, unfortunately, will have to be revised; as too much was undertaken to be thoroughly done in the time available. As these old species are more fully

known and recognized, a great number of our recent so-called new ones will fall into synonymy. It would advance systematic mycology more to re-describe, illustrate, and make well known, one of the uncertain older species than to describe a so-called "new species" based, too often, on insufficient material and study, and which may already have several names.

In speaking of descriptions, we have gone from one extreme to the other; from the one or two line descriptions of some of the older mycologists to the one or two page descriptions of the more recent. A description need not be long in order to give a clear and definite idea of a species. What is needed is a concise and accurate description of the essential features of the organism, emphasizing the particular characters which separate it from its nearest relatives. In order to compare the different characters readily the different parts of the fungus should always be described in the same order. In some instances, one finds emphasized as a specific character of a new species one not mentioned in the description of its nearest relatives. Is one to infer that this character is lacking in the other species or that it is different? There is little satisfaction or consolation to find at the end, in connection with the description of a new species in which no particular specific distinction is pointed out, the note: "This is a well marked species easily distinguished from its relatives."

In addition to adequate descriptions good illustrations should be given wherever possible. The best descriptions and illustrations, however, do not always make possible certain identification of a species. Cotype or authentic specimens should be made available. As already stated, the material upon which a species is based should be ample and portions of it should be deposited in the large herbaria for comparison by monographers. The usual excuse for not doing this is the scantiness of the type material. If the material is too poor or scanty for division, this would, in most cases, be sufficient reason for withholding the publication; as it would only add one more to the great mass of uncertain species already existing. Since, in the present state of our knowledge, genera and species of fungi are largely mental concepts; it is best from a practical as well as a scientific standpoint to be conservative in our concepts and interpretations and to follow a median course, rather than that of

the so-called "lumpers" or "splitters." In this connection the need for more careful and much more thorough field studies and collections should be emphasized. One who has not examined the collections in our larger herbaria, either in this country or in Europe, would scarcely believe how few and poor are the specimens of many of even the common species. In most cases it seems to be taken for granted that if the species is a common one two or three specimens in a herbarium are sufficient. This is a great mistake. Many of our common species are very variable and in some cases, perhaps, two or more are confused under a single name, so that abundant and numerous gatherings are necessary for an adequate study of the group. A large quantity of much better material from a wider range of localities must also be accumulated before satisfactory monographic work can be done. In this connection I would call attention to the desirability of aiding, encouraging, and developing a much larger number of amateur collectors and students of fungi. At present the race seems to be nearly extinct in this country. Amateurs may be of the greatest assistance in aiding and advancing systematic studies of fungi by supplying taxonomists and herbaria with good specimens and field notes on the species growing in their localities, and at the same time they may derive a great deal of pleasure and recreation from the work.

In this connection I may repeat what I have said before; that one of the greatest discouragements to amateur botanists is the frequent change of generic and specific names. Fortunately, thus far, no very widespread and serious attempts have been made to change our fungus names on a strict priority basis, and we know of nothing that would interfere more with the advancement and popularization of systematic mycology than a general attempt to apply this plan to the names of fungi; as it would result in a change of a great many of our best known names of genera and species. The names at present in general use should be conserved, if we are ever to have a reasonably uniform and stable nomenclature. In this connection we may also call attention to the present tendency to use outlandish and sesquipedalian names. Names are for convenience and practical purposes and should therefore be as short and suggestive as possible. Some of the

generic and specific names which are being perpetrated at present are indefensible and intolerable, and though they may have some temporary recognition they will, I believe, eventually be discarded. Consider the following mild examples: *Chaetobasidiella vermicularioides, Ectotrichophyton mentagrophytes* and *Methysterostomella argentinesis.* Another need for the advancement of systematic mycology is that of centralized publication of taxonomic work and especially of descriptions of new genera and species. If such material could be published in a single journal in each country, it would be a great step in the direction of economy and efficiency, and a great saving of time and labor for all concerned.

Another need is more life history studies. Recent work has shown that such studies may throw much light upon the origin and relationships of different groups. Such work also reveals the fact that this line of investigation does not give promise of providing an easy solution to our taxonomic problems; as species showing close relationship according to their perfect stages are sometimes found to have quite diverse conidial forms and vice versa.

The study of heterothallism and hybridization has also opened up a vast field for investigation and shows the possibilities of complications as to the origin and systematic relations of many groups, especially the Ascomycetes.

In connection with life history studies, in many cases efforts to obtain all the spore stages of an organism in pure culture have failed. In some of these instances, we know that failure has been due to heterothallism. In many other cases, however, failure is probably due entirely to lack of providing natural conditions in the way of nutrition and environment for the fungus. If we could successfully reproduce natural conditions in the laboratory or in the open, under control, there is every reason to believe that success would be attained.

REWARDS

No one needs reminding that Mycology is not a road to wealth, and that large stipendiary emoluments are hardly to be expected. However, a real mycologist, as any other laborer, is worthy of his hire. Just at present, the demand for professional mycologists is not great, but those who are impelled by a deep desire for knowledge of this subject and who have great patience and perseverance,

will find some way of securing a livelihood and the comfort to be derived from knowing that one is contributing to the advancement of knowledge and to a broader appreciation and understanding of the wonders of Nature.

We have already emphasized the fact that mycological work demands persistence, patience, and accurate observation; careful experiments, and painstaking records and measurements. The study of mycology, however, has other phases than these more or less impersonal ones which are pursued with scientific detachment and freedom from imagination, sentiment or emotion. The mycologist may and should enjoy the beauties and marvels of nature and speculate about her mysteries without interfering with the accuracy of his observations or the strict and logical interpretation of his results. In other words, there is no reason why the "fun" should be taken out of fungi. The joy of discovery and the intellectual pleasure to be derived from the contemplation and appreciation of the wonderful variety of forms, their complexity of structures and the mysteries involved in their origin and evolution, their life histories and relationships, and from the discovery of unknown facts and forms—these are some of the richest rewards for the student.

Mycologists, as others, sometimes have their pessimistic moods. Tulasne tells us that Fries on such an occasion said: "Mycology is one of those despised and neglected studies which bring their pursuers neither money nor glory." As most of us know from experience, however, it may bring something much more satisfying and valuable than either money or glory. To quote the poet: "To him who in the love of nature holds communion with her visible forms she speaks a various language." For inspiration and encouragement one should read the lives of such men as Darwin, Huxley, Wallace and others. Much good advice may be obtained from reading the introduction to Tulasne's classic work recently translated. He says: "If the mycologist of today desires as is fitting nothing more than to please and at the same time provide the reward, let him throw away every fiction contrary to nature and understand that he has reached the height of virtue and glory, when by hard labor and wise study he has attained the truth and has revealed it in full daylight."

Probably in no branch of biology is there a broader field and greater opportunity for discovery and contribution to knowledge than in Mycology. It not only furnishes an extensive field for scientific discovery, recreation, and pleasure, but great opportunity for practical application in agriculture, the arts and industry. All the time at my disposal might have been spent in listing the most important economic applications and aspects of the subject.

Let us therefore continue the good work with higher ideals and greater zeal, relying upon the assurance that Mother Nature will do for us what she did for Agassiz:

> "Whenever the way seemed long,
> Or his heart began to fail,
> She would sing a more wonderful song,
> Or tell a more marvellous tale."

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

A NEW SPECIES OF LEPIOTA [1]

S. M. Zeller

(with plate 26)

This mushroom is one of the very first to make its appearance after the early fall rains. It is one of the most common Agarics of the fall season to be found throughout the Willamette Valley. *Lepiota Barssii* grows in locations similar to those where the smooth *Lepiota* (*L. naucina*) is found, the two sometimes near each other under the same ecologic and climatic conditions. *L. Barssii* generally comes out a few days earlier than *L. naucina* but the latter may be found for a considerable time after *L. Barssii* has disappeared.

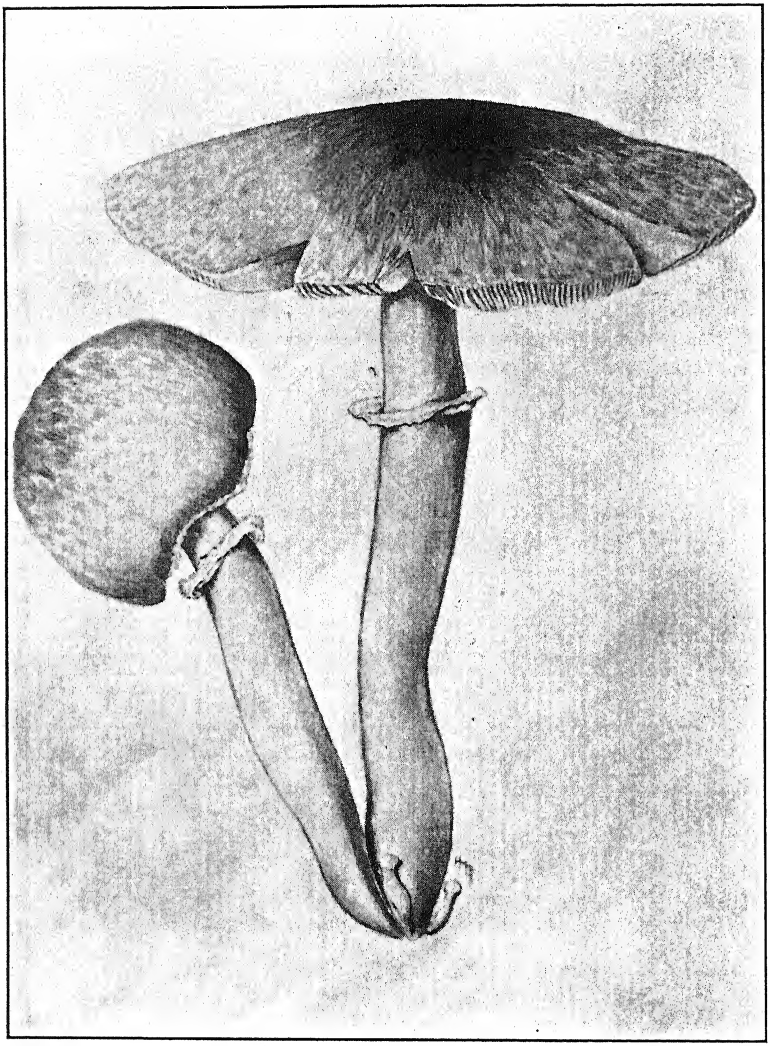
L. Barssii occurs in pastures, plowed fields and gardens, or in stubble (grain) fields. Its fructifications thrive and grow to their largest size around old straw stacks or manure piles. A favorable habitat is strawberry or potato plantings.

In favorable locations this mushroom may be collected in large quantities and it has proven to be very palatable. For many years it has been collected by mycophagists without discrimination from *L. naucina,* with which it compares very favorably as an edible mushroom.

This mushroom is a beautiful *Lepiota* belonging to the group *Procerae-annulosae,* as described by Kauffman.[2] It is large and of stately form, as illustrated in plate 26, a photograph of a water-color painting by Dr. Helen M. Gilkey. *L. Barssii* is perhaps more closely related to *L. naucina* than to other species of the genus, but is easily distinguished by the characteristically gray color and scaly surface of the pileus. In *L. naucina* the gills are more nearly equal, then slightly narrowed behind, sometimes almost sinuately indented, and of much softer texture than those of the same age in *L. Barssii.* The stem of the latter is not enlarged at the base as in *L. naucina.*

[1] Published as technical paper No. 210 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

[2] Kauffman, C. H. The genus *Lepiota* in the United States. Mich. Acad. Sci. Papers **4**: 311–344. *illus.* 1924.



Lepiota barssii

The writer takes pleasure in dedicating this species to his botanical colleague, H. P. Barss, Head Professor of Botany and Plant Pathology, Oregon State Agricultural College. Professor Barss was one of the first to notice the characters by which this species is distinguished from *L. naucina*. The diagnostic description follows:

Lepiota Barssii sp. nov.

Gregaria vel caespitosa: pileo carnoso, 7–15 cm. lato primito subgloboso vel ovoideo dein convexo vel plano-expanso obtuso-umbonato vel subumbilicato; margine interdum radiatim rimoso; superficie arida fumoso-grisea vel "drab," [3] disco fuscidiore, fusco vel "Cinnamon drab," [3] squamulis fibrillosis fuscis vestito; contextu primito albo dein sordido postice crasso margine pertenui, odore et sapore grato; lamellis 7–16 mm. latis inaequalis postice tenuitiore subconfertis liberis albis mutans straminescens, acie levis sterilis; stipite 8–18 mm. crasso 8–12 cm. longo subaequali, farcto dein cavo, glabro vel sericeo albo; annulo amplo supero collarioideo saepe mobili persistenti albo, extus laminis stipitis et veli efformato; sporis ellipsoideis-ovoideis levibus albis guttulatis, magnitudinis variabilis 7.5–9.5 × 5–6 μ.

Ad terram in pratis et in horto, Oregon occid., Amer. Bor.

Gregarious or caespitose; *pileus* 7–15 cm. broad, fleshy, at first subglobose to ovoid, then convex to plano-expanded, obtusely umbonate to subumbilicate, sometimes splitting radially at margin, *surface* dry, smoke-gray to drab with darker, fuscous or cinnamon darb umbo, covered by fibrillose, fuscous scales; *flesh* at first white, then sordid, thick at disk but very thin toward margin; *gills* 7–16 mm. broad, unequal, narrower behind, close but not crowded, free, edge even, sterile, white changing slightly stramineous; *stem* stout, 8–18 mm. broad above, 10–15 mm. broad below, 8–12 cm. long, almost equal, stuffed then hollow, glabrous or silky above and below the ring, white within and without; *annulus* formed from veil and outer layer of stem, white, collar-like, persistent, superior, often movable at maturity; *spores* ellipsoid-ovoid, variable in size in same plant, 7.5–9.5 × 5–6 (ave. 7.7–5.2) μ, smooth, white, guttulate; *odor* and *taste* pleasant.

In pastures, plowed fields, or gardens, September. Very common throughout the Willamette Valley, western Oregon.

OREGON STATE AGRICULTURE COLLEGE,
CORVALLIS, OREGON.

EXPLANATION OF PLATE 26

Lepiota Barssii. Photograph of a water-color painting by Dr. Helen M. Gilkey.

[3] Ridgway, Color Standard.

THE HYDNACEAE OF IOWA. III. THE GENERA RADULUM, MUCRONELLA, CALDESIELLA AND GLOIODON

L. W. MILLER

(WITH PLATE 27)

RADULUM Fries, Elench. Fung. **1**: 148. 1828.

Resupinate or rarely reflexed, ceraceous; teeth blunt, generally coarse, deformed, irregularly scattered or confluent. Growing on wood.

KEY TO THE SPECIES OF RADULUM

1. Narrowly reflexed, sometimes resupinate, thick, not cracking; clamp connections numerous; spores 5–7 × 3–4 μ.............3. *R. pallidum.*
1. Resupinate, adnate, varying in thickness, sometimes cracking; clamp connections present or absent; spores usually larger.................(2)
   2. Cracking; clamp connections few or absent; spores 6–8 × 3–4 μ 2. *R. quercinum.*
   2. Generally not cracking; clamp connections numerous; spores 8–12 × 3–4 μ, curved..................................1. *R. orbiculare.*

1. RADULUM ORBICULARE Fries, Elench. Fung. **1**: 149. 1828. (PLATE 27, FIG. 1.)

Hydnum Radula Fries, Syst. Myc. **1**: 422. 1821.
Sistotrema Radula Pers. Myc. Eu. **2**: 195. 1825.

Resupinate, orbicular, becoming confluent, soft ceraceous, light ochraceous-buff; margin similar or byssoid and white; teeth variable, cone-shaped to cylindrical or plate-like, obtuse, scattered or fascicled; hyphae 2–4 μ in diameter, distinct, with numerous clamp connections, not arranged in distinct zones; basidia 20–30 × 5–6 μ, clavate, with 4 sterigmata; spores 8–12 × 3–4 μ, cylindrical, curved, smooth, hyaline.

The large, curved, cylindrical spores clearly separate *Radulum orbiculare* Fries from the other Iowa species of *Radulum.* This species seems close to *Radulum hydnans* Schw. and *Corticium colliculosum* Berk. & Curt. Burt regards these latter two as synonyms and belonging in the genus *Corticium,* and therefore

distinct from *Radulum orbiculare.* He states that *Corticium hydnans* (Schw.) may be distinguished in doubtful cases from *Radulum orbiculare* Fries by the absence of gloeocystidia. I have examined many specimens of *Radulum orbiculare* from Europe and several so determined by Burt and was unable to find definite gloeocystidia in any of these. The spore size as recorded by Burt is considerably smaller for *Corticium hydnans.*

Fairly common in Iowa. Collected on deciduous wood from April to September. Reported from Washington and from many regions in the central and the eastern United States, including Iowa.

2. RADULUM QUERCINUM Fries, Hymen. Eu. 623. 1874. (PLATE 27, FIG. 3.)

Hydnum quercinum Fries, Syst. Myc. **1**: 423. 1821.
Hydnum fagineum Pers. ex Fries, Syst. Myc. **1**: 433. 1821.
Sistotrema fagineum Pers. Myc. Eu. **2**: 194. 1825.
Radulum fagineum Fries, Elench. Fung. **1**: 152. 1828.

Fructification resupinate, orbicular then confluent and effused, sometimes subdecorticating, crustaceous-ceraceous, adherent, often cracking in drying, cinnamon-buff to clay color; margin thin, similar or slightly villose, usually lighter in color; teeth variable, short and obtuse to long, cylindrical and slightly pointed; hyphae 2–5 μ in diameter, mostly thin-walled, with few clamp connections; basidia clavate, with 2–4 sterigmata; spores $6–8 \times 3–4\ \mu$, ellipsoid to short cylindrical, depressed laterally, smooth, hyaline.

The European descriptions of *Radulum quercinum* Fries indicate a fungus very near to *Radulum pallidum* Berk. & Curt. Six specimens of the former species from Litschauer, Bourdot and Bresadola were examined at the New York Botanical Garden and the Farlow herbarium. These seemed to be distinct. The hyphae lack the characteristic clamp connections and the spores are slightly longer. The thin, adnate, resupinate fructification is usually cracked to the substratum. There is little suggestion that this type of fructification may occur reflexed as in *Radulum pallidum.*

Rare in Iowa. Collected in October on deciduous wood. Schweinitz reports the occurrence of *Hydnum quercinum* Fries from Pennsylvania.

3. RADULUM PALLIDUM Berk. & Curt. Grevillea 1: 145. 1873. (PLATE 27, FIG. 2.)

Resupinate to narrowly reflexed, tomentose and white on the upper surface; orbicular at first, then confluent and slightly effused, adnate, ceraceous, thick, usually not cracking, pinkish buff to vinaceous-buff and vinaceous-fawn; margin tomentose, white; teeth variable, short, obtuse, smooth or slightly fimbriate, often confluent in irregular groups; hyphae 2–4.5 μ, distinct, with numerous clamp connections, more or less parallel along the substratum and ascending obliquely to the compact hymenium, hyaline or granular; basidia $15–35 \times 4–7\ \mu$, clavate, with 4 sterigmata; spores $5–7 \times 3–4\ \mu$, ellipsoid, obliquely attenuated, slightly depressed laterally, smooth, hyaline.

This species resembles *R. quercinum* Fries as that species is known in Europe. It seems to differ in its often reflexed margin, the abundance of clamp connections and slightly smaller spores. Resupinate specimens usually can be distinguished by the vinaceous tinge of the hymenium, the thicker and less cracked fructification and the more abrupt, tomentose margin. These macroscopic characters are exceedingly variable, however, as is true of other species of *Radulum*. This species seems also to be known as *Radulum orbiculare* Fries in this country, judging by the many herbarium specimens so referred. Lloyd clearly and accurately separated the two in his paper, *The genus Radulum,* 1917. Later (Myc. Writ. 1079. 1921) he considered *R. pallidum* merely the American representative of *R. orbiculare*. The longer, curved spores, the invariably resupinate fructification and the softer, ceraceous texture seems clearly to separate *R. orbiculare*. Iowa specimens are identical with a specimen at The New York Botanical Garden which Banker has compared with the type. They also agree with Lloyd's material.

Abundant on decaying wood and bark of oak and other frondose species, often on charred wood; collected throughout the year. Its occurrence is widely reported from the central and eastern states.

MUCRONELLA Fries, Hym. Eu. 629. 1874.

Subiculum absent or consisting of a floccose, fugacious mycelium; spines subulate, entire. Growing on wood and bark.

KEY TO THE SPECIES OF MUCRONELLA

1. Spines gregarious but not fascicled; spores 4–7 × 2–4 μ..1. *M. aggregata.*
1. Spines in fascicles of 2–8; spores 14–16 × 10–12 μ..........2. *M. Ulmi*

1. MUCRONELLA AGGREGATA Fries, Monog. Hymen. Suec. 2: 280. 1863. (PLATE 27, FIG. 7.)

Subiculum absent or consisting of a few spreading hyphae; spines 0.5–1.5 mm. in length, subulate, entire, acute, gregarious, in groups, reported white when fresh, chamois in the herbarium; cystidia absent; hyphae 2–4 μ in diameter, sometimes 6–8 μ in the interior of the spine, thin-walled, with few clamp connections, accompanied by calcium oxalate crystals; basidia 10–16 × 3–5 μ, clavate; spores 4–6.5 × 2.5–3.5 μ, ellipsoid, smooth, hyaline.

This species is recognized by the gregarious spines which are more or less distinctly separated from each other. It seems closely related to *Mucronella calva* (Alb. & Schw.) Fries, *M. minutissima* Peck., *M. abnormis* P. Henn., and *M. ramosa* Lloyd. There seems to be little difference in the microscopic structure of these species as described in the literature. A speciment labeled *M. calva* (Alb. & Schw.) from the herbarium of Bresadola at The New York Botanical Garden is identical with *M. aggregata* as here understood. Lloyd (1922) states that *M. ramosa* "is similar to *M. aggregata* except the separate plants appear as if branched." An old specimen (No. *265*) in the University of Iowa herbarium answers very well to Lloyd's description and figure (*Fig. 2036*) of *M. ramosa* but is not sufficiently distinct from *M. aggregata* to justify specific rank.

Three specimens of *M. aggregata* have been collected in Iowa. On decaying wood in November. Its occurrence in Maine, New York, Ohio and Iowa has been reported.

2. MUCRONELLA ULMI Peck, Ann. Rep. N. Y. State Mus. **54**: 154. 1901. (PLATE 27, FIG. 4, 5.)

Subiculum absent; spines 1–3 mm. in length, 0.2–0.35 mm. in diameter, in fascicles of 2–8, rarely single, terete, subulate, acute, soft, curved, dusty dull violet with a white base, soon becoming white and mealy; hyphae 2.5–3 μ in diameter, not incrusted, walls thickened, with few septa and clamp connections, hyaline; basidia large, 30–35 × 10–15 μ, clavate, with 4 sterigmata, accompanied by slender, hyaline and slightly projecting, paraphysoid hyphae;

spores 14–16 × 10–12 μ, obovate, smooth, with a prominent apiculus, hyaline.

This species is recognized by its fascicled spines, its violet color and the large spores.

In the original description of *M. Ulmi* the spines are recorded as greyish or pallid. Peck does not refer to the spore characters. Overholts (1920) made some additional notes on the same species. He described the fructification as white, drying gray but was unable to obtain spores. Mention was made of specimens with a lavender or purplish tint. A fungus has been collected repeatedly in Iowa to which the descriptions of Peck and Overholts closely apply. The spines when fresh are usually distinctly violet in color and large, lemon-shaped spores are produced. Several specimens were collected which had whitish spines but these were assumed to have faded. Recently the type of *M. Ulmi* in the New York State Museum at Albany was examined and found to be unquestionably the same species. The type specimen has whitish spines and the large characteristic spores.

This fungus is very inconspicuous but apparently common in Iowa. Collections were made from June to November on the bark of both living and dead trunks of willow, oak, ash and elm. The occurrence of *M. Ulmi* seems to be recorded only from the type locality in New York and from Pennsylvania by Overholts.

CALDESIELLA Sacc. Michelia **1**: 7. 1877.

Resupinate, soft, floccose, dark; spores subspherical, colored. Growing on wood.

CALDESIELLA FERRUGINOSA (Fries) Sacc. Michelia **2**: 303. 1881. (PLATE 27, FIG. 6.)

Hydnum ferruginosum Fries, Syst. Myc. **1**: 416. 1821.
Hydnum ferrugineum Pers. Myc. Eu. **2**: 189, *non* Fries. 1825.
Hydnum crinale Fries, Epicr. 516. 1838.
Acia ferruginea (Pers.) Karst. Bidr. Finl. Nat. Folk **37**: 112. 1882.
Odontia barba-jovis Pat. Tab. Fung. **3**: 110. 1884.
Hydnum tabacinum Cooke, Grevillea **14**: 129. 1886.
Phaeodon tomentosus Schrad. ex Schröt. Krypt.—Fl. Schles. **3**[1]: 458. 1889.

Acia tomentosa Schrad. ex Karst. Bidr. Finl. Nat. Folk **48**: 362. 1889.

Odontia crinalis (Fries) Bres. Atti Accad. Rovereto **3**: 96. 1897.

Odontia ferruginea Pers. ex Banker, Bull. Torrey Club **29**: 439. 1902.

Caldesiella crinalis (Fries) Bourd. & Galz. fide Rea, Brit. Basid. 651. 1921.

Resupinate, effused, floccose, slightly separable, ochraceous-tawny to mummy brown; margin similar or slightly lighter in color; spines subulate, conical, acute, terete, 2 mm. or less in length; hyphae 2–5 μ in diameter, loosely interwoven and in slender branching strands in the subiculum, with numerous clamp connections, mostly dark colored; basidia 40–60 $\times$ 6–8 μ, cylindrical, with 2–4 prominent sterigmata measuring 5–8 μ in length, becoming colored; spores 8–10 $\times$ 7–9 μ, subspherical, tuberculate, benzo brown in mass.

This species is well marked. The dark-colored, tomentose, resupinate fructification and the large, dark, tuberculate spores are distinctive characters.

A number of specimens were collected in Iowa from the same log from August to November in 1931 and 1932. This log was lying in an open pasture which had recently been cleared. When first collected the fungus covered a considerable area of the underside of the log and crept over about 8 square inches of the ground immediately under the log. The portion on the ground bore distinctly upright spines as well as spines appressed somewhat to the subiculum. A small specimen was also collected at Milford, Iowa in 1932. Apparently uncommon in Iowa. It is known from California and at least eight central and eastern states. A fragment of the type of *Hydnum tabacinum* Cooke, a specimen of *Hydnum crinale* Fries from the herbarium of Fries, and a specimen of *Odontia crinalis* (Fries) from Bresadola at The New York Botanical Garden seem to be identical with specimens collected in America.

GLOIODON Karst. *emend.* Banker, Mycologia **2**: 10. 1910.

Resupinate or pileate and laterally sessile, tough, dark, consisting of branched processes in a coarse tomentum; spores faintly roughened, short elliptical, hyaline. Growing on wood.

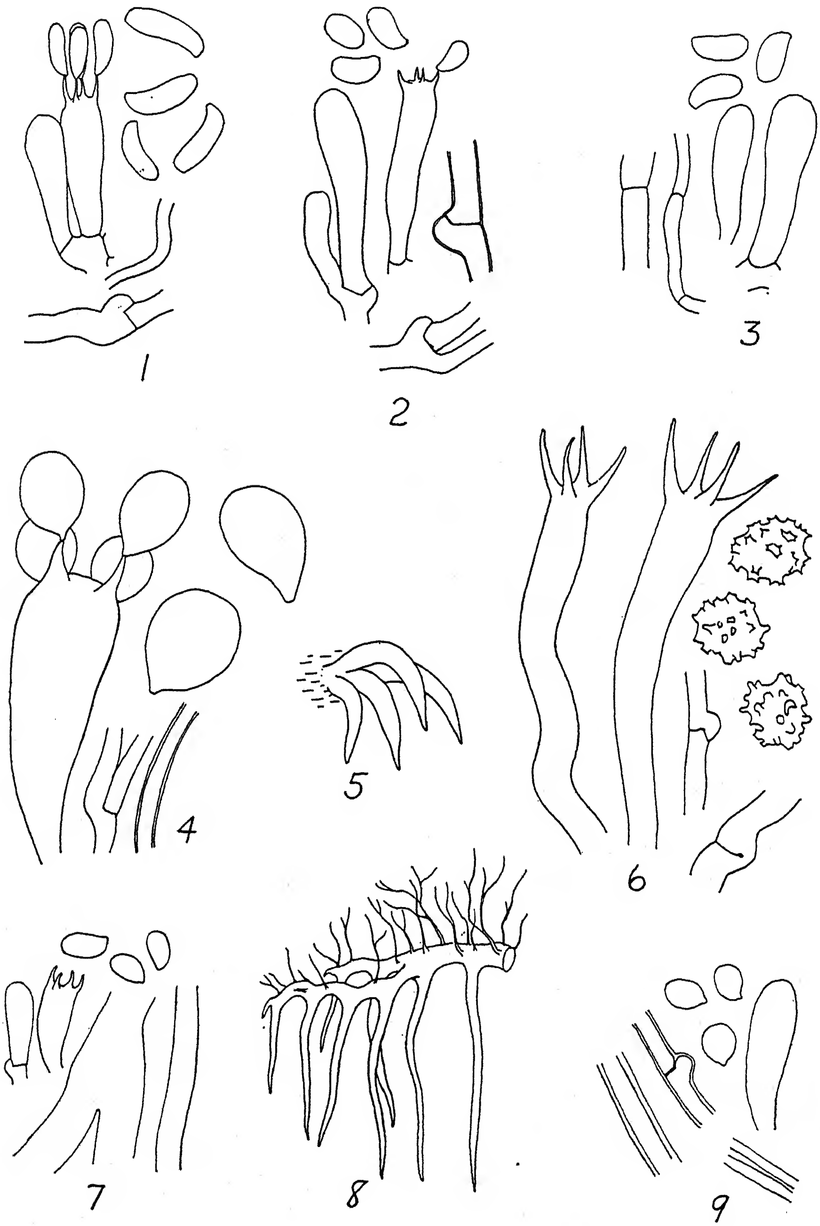
GLOIODON STRIGOSUS (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. **5**: 28. 1879. (PLATE 27, FIG. 8, 9.)

Hydnum strigosum Swartz ex Fries, Syst. Myc. **1**: 414. 1821.
Hydnum stratosum Berk. Lond. Jour. Bot. **4**: 307. 1845.
Sclerodon strigosus (Fries) Karst. Bidr. Finl. Nat. Folk **48**: 361. 1889.
Mycoleptodon strigosum (Fries) Pat. Tax. Hymén. 117. 1900.
Leaia piperata Banker, Mem. Torrey Club. **12**: 175. 1906.
Leaia stratosa (Berk.) Banker, Mem. Torrey Club. **12**: 177. 1906.
Gloiodon stratosus (Berk.) Banker, Mycologia **2**: 11. 1910.

Fructification resupinate to reflexed or dimidiate, occasionally stratose from successive growths, 5 mm. or less in thickness, dry, tough, fibrous, cinnamon-brown, consisting of flexible, repeatedly branched processes which are partially submerged in a dense, coarse tomentum; margin fimbriate from the projecting ends of the branches or tomentose; spines 3 mm. or less in length, 0.2 mm. or less in diameter, slender, terete, acute, arising from the branched processes which they resemble in texture, mummy brown with a thin, light mineral gray surface layer when dry; hyphae 2.5–5 μ in diameter, septa widely separated, with clamp connections in the mycelial strands, faintly colored; basidia clavate; spores 4.5–5.5 $\times$ 3.5–4 μ, subspherical to elliptical, faintly roughened, hyaline.

This species may be recognized by the layer of ramifying processes which support the spines below and the dense tomentum above. It is reported as having an intensely acrid taste.

In 1897 Bresadola indicated that *Hydnum strigosum* Fries, applied to a pileate form, was identical with *Hydnum stratosum* Berk. which was based on a stratose, resupinate specimen. Banker (1906) apparently was not familiar with Bresadola's paper and had not seen authentic or type material. He applied *Steccherinum strigosum* to an entirely different fungus. He recognized *Hydnum* (*Leaia*) *stratosum* Berk. and applied the new name *Leaia piperata* to pileate forms of the same species. Later (1910, 1913) Banker became familiar with the true *Hydnum strigosum* and reported having been the types of the species concerned. Consequently, the specific name *strigosum* was employed rather than *piperata* for the pileate forms. He again regarded the resupinate speci-



HYDNACEAE

mens and the pileate specimens as two distinct species. I have examined Banker's material and the type of *Hydnum stratosum* Berk. at The New York Botanical Garden and am convinced that Bresadola's conclusions are correct.

Collected once in Iowa by Holway. The specimen is in The New York Botanical Garden. Its occurrence in Iowa is also reported by Cejp but the specimen in the University of Iowa herbarium so determined by him is *Irpex pachyodon.* Reported from seven eastern states. Apparently rare.

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IOWA CITY, IOWA

EXPLANATION OF PLATE 27

All figures, except 5 and 8, drawn with camera lucida at a magnification of 1650 diameters, reduced to × 1000 in reproduction. Hyphae, basidia and spores are shown in each figure except 5 and 8.

Fig. 1, *Radulum orbiculare;* 2, *R. pallidum;* 3, *R. quercinum;* 4, *Mucronella Ulmi;* 5, *M. Ulmi,* habit sketch; 6, *Caldesiella ferruginosa;* 7, *Mucronella aggregata;* 8, *Gloiodon strigosus,* a portion of a branching process showing the position of the teeth below and the brownish tomentum above; 9, *G. strigosus.*

THE DEVELOPMENT OF CORDYCEPS AGARICIFORMIA [1]

Wilbert A. Jenkins

(with plates 28 and 29 and 1 text figure)

INTRODUCTION

The members of the genus *Cordyceps* constitute a unique group of approximately two hundred species. With the exception of two species (*Cordyceps agariciformia* (Bolt.) Seaver and *C. parasitica* (Willd.) Seaver) which attack the hypogeous fruits of the fungus *Elaphomyces,* all these species occur as parasites on insects or spiders.

Our knowledge of the biology of *Cordyceps* is essentially limited to early studies of entomogenous species (de Bary, **4**, **5**; Tulasne, **40**; Sopp, **39**; Atkinson, **1**; Pettit, **36**). These investigators confined their studies to a single stage in the cycle, principally the development of the perithecium and ascospores (Fisch, **15**; Lewton-Brain, **29**; Maire, **30**) because they lacked sufficient material to study the entire cycle of development or because they were handicapped by the lack of suitable microtechnical methods. Recently, Varitchak (**41**, cf. also Varitchak, **42**) contributed to our knowledge of the morphological and cytological development of one of the entomogenous species. Facts established from studies on supposedly closely allied genera (Vincens, **45**; Killian, **25**; Gäumann, **17**) have been employed as a means of arriving at an understanding of the genus *Cordyceps.*

In the present investigation an attempt is made to follow the course of development, both morphological and cytological, of *C. agariciformia* from the period extending from the sclerotial stage to the maturation of the ascospores. There has been to date only one such investigation (Varitchak, **41**) and since this work was

[1] A revision of a dissertation submitted to the Board of University Studies of the Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy, June, 1933.

done on an altogether different species, the present contribution should afford opportunity for comparison and contrast.

MATERIALS AND METHODS

The material of *Cordyceps agariciformia* consisted of clavae ranging in stages of development from those which had emerged to a height of only a few millimeters above the parasitized *Elaphomyces* fruits up to and including clavae which bore mature perithecia and spores. The first collection of material was made at Gainesville, Florida, fixed in Gilsons fluid on February 24, 1924 and sent to the writer in November, 1932. The second collection included a rather complete series of developmental stages and was received in living condition from Cocoa, Florida on January 17, 1933. The third collection consisted of more mature stages and was sent from Gainesville on February 4, 1933. This living material, on arrival, was still attached to its host and was apparently healthy. The material was immediately washed in running water and fixed in formalin-acetic-alcohol, care being taken to cut the larger clavae into appropriate sizes to permit rapid penetration of the fixative. Due to the presence of adhering and incorporated sand particles on the *Elaphomyces* fruits and in the very young clavae, it was found necessary to desilicify these, after fixation, with hydrofluoric acid. The material was then dehydrated and embedded in paraffin. Sections were cut five, eight and ten microns in thickness in transverse, radial and tangential planes.

Various stains and combinations of stains were used, as Gram's Gentian violet (Couch, **10**), Heidenhain's iron alum haematoxylin in combination with fast green, the same in combination with orange III in clove oil, safranin alone and Heidenhain's iron alum haematoxylin alone. While Gram's stain proved very useful in differentiating the structure of the opercula of the asci, walls of the asci and vegetative mycelium, and especially as a differential stain to show the relation of the mycelium to that of *Elaphomyces*, Heidenhain's iron alum haematoxylin used alone proved superior for the finer details of nuclear structure. The sections were in all cases cleared in clove oil, and in most cases this was followed by a treatment with cedar oil before passing them into xylol and mounting in balsam. The haematoxylin was applied in general

according to the schedule of Gwynne-Vaughan (Gwynne-Vaughan and Williamson, 20) though for some stages shorter periods of staining were found to be adequate.

HOST RELATIONS

DeBary (4, 5) has given a remarkable account of the phenomena attendant on infection, the formation and circulation of endogenously produced conidia in the "blood stream" and their germination to form sclerotia in the bodies of larvae of *Gastropacha Euphorbiae*, by the entomogenous species, *Cordyceps militaris*. Sopp (39) found that *C. norvegica* Sopp is normally a saprophyte on the forest floor, its parasitism on *Gastropacha Pini* being facultative. The results of his numerous inoculation experiments confirmed those of de Bary, e.g., that a given species of *Cordyceps* is not necessarily confined to a given species, genus or even group of insects. Lewton-Brain (29) reported the occurrence of the parasitic hyphae of *C. ophioglossoides* (Ehrh. Link (*C. parasitica* (Willd.) Seaver) in close contact with, and in some cases actually cemented to, the hyphae of *Elaphomyces*. In such cases a thin spot was apparent on the *Elaphomyces* hypha opposite the point of contact with the hypha of the parasite. Cells were likewise seen that had been penetrated by the parasite but no well-defined haustoria were seen. Lewton-Brain also reported the presence of isolated groups of cells of the host scattered about in the base of the clava of the parasite.

Within the base of the clava proper, in young clavae, the writer noted that the isolated groups of host cells were rather completely destroyed but in the surrounding areas the host cells were in various states of disorganization. In a few instances the hyphae of the parasite could be traced for considerable distances and in such cases a clearer picture of their activity could be formed. Although the point could not be conclusively proved with this material, there is considerable evidence that the *Cordyceps* mycelium is, in the early stages of its parasitism, an interhyphal parasite. A few favorable sections showed that narrow hyphae, much narrower than the parent hyphae, had penetrated the host cells (PLATE 29, FIG. 43). Whether these hyphae could be regarded as haustoria is, of course, open to debate. In such cases the cytoplasmic

contents of the host cells had not been completely destroyed. Other observations showed the mycelium of the parasite to be definitely intrahyphal, but in such cases the cytoplasmic contents of the host cells were almost or quite completely destroyed (PLATE 29, FIG. 45). Such a relation would be, if substantiated, entirely in keeping with the relation existing between many fungous parasites and their hosts.

Evidence indicates, likewise, that the clava of this species has its origin from numerous hyphae which arise as products of germination of certain cells of the pseudosclerotium situated just beneath the cortex of the *Elaphomyces* fruit body. This type of initiation has been described for *Claviceps purpurea* (Killian, **25**) and *C. microcephala* (Vincens, **45**). Also observations by the writer on the origin of the clavae of *Cordyceps clavulata* (Schw.) Ellis & Ev. show that in this species also the clava arises in the same manner as has been described above.[2] Should these observations prove to be correct in the case of *C. agariciformia,* Lewton-Brain's comparison of the base of the clava to the 'foot' of the *Anthoceros* or of the fern embryo, and his statement to the effect that the tissue of the host is destroyed and replaced by the "advancing 'foot' of the *Cordyceps*" are unwarranted. In the light, then, of evidence obtained from *Claviceps, Cordyceps clavulata,* various other entomogenous fungi (Petch, **35**; Massee, **31**; Cooke, **9**; Vincens, **43**) and indeed a majority of fungous parasites it is most probable that the parasite established its relation with the host long before clava formation began and has long since assimilated an abundance of food and stored it within the cells of its mycelium in preparation for its reproductive processes.

THE FORMATION AND STRUCTURE OF THE CLAVA

The earliest stages studied consisted of young clavae, a few millimeters long, which were sectioned longitudinally. Such sections showed very clearly that the emerging hyphae had ruptured the cortex of the host and in so doing had left groups of broken host cells scattered among the hyphae of the clava. Just how this rupture was accomplished is not known, but the evidence available indicates that the mechanics of the process are similar to that

[2] Paper to be published at a later date.

found in *C. clavulata*. In the latter, the hyphae arising from the germination of the sclerotial cells just beneath the integument of the host apply their tips against the cuticular covering and work their way through by dissolving a passageway or by mechanical pressure or by a combination of both processes. In other cases, individual hyphae emerge through natural openings in the cuticle. Though the hyphae emerge singly at first, they always emerge in definite groups so that the cuticular covering of the insect is riddled like a sieve at various points over its surface. Ultimately the continued pressure from the growth of other hyphae pushing up through these groups ruptures the cuticle at various points and the clavae emerge as cushion shaped structures, quite compactly organized toward the center, but of a loose, even floccose organization along the periphery. Bits of the cuticle which remain lodged among the hyphae of the clavae remain in the same relative position due to the predominant apical growth of the hyphae, so that later these fragments of cuticle are seen scattered among the hyphae at the base of the clavae.

In the young stages of *C. agariciformia*, the clava was composed of slightly larger and more compacted hyphae along the central axis. In sections of somewhat older specimens, ten to eleven millimeters long, this central region was composed of distinctly larger, longitudinally coursing hyphae intertwined with other very narrow ones. At the periphery, especially near the apex, the central region had given rise to a thin external layer of exceedingly fine and intricately interwoven hyphae which formed the outermost covering of the clava. In longitudinal sections of this stage, even prior to staining, the hyphae of the central region seem to form the skeleton that gives rigidity to the clava. Otherwise there was no differentiation of parts either as regards color or structure in the clavate, opaque head. This early differentiation was not noted in *C. militaris* (Varitchak, **41**).

It was at first thought that the fertile hyphae which ultimately initiate the perithecia might possibly be differentiated in the very young clava, even before emergence from the *Elaphomyces* fruit body. Accordingly, the younger stages of clava formation were all cut in median longitudinal sections, in the hope of finding fertile hyphae differentiated in the pseudosclerotium. Careful examina-

tion of all sections of this stage of development failed to give any evidence of such differentiation, nor could the hyphae from which the fertile hyphae arose during later stages of clava formation be traced for any considerable distance due to the interweaving of the hyphae with each other. These longitudinally cut sections were especially fortunate in another respect, however, for they showed the relative position, the relative time of origin and the relative rapidity of development of perithecia all in one section. Sections of clavae ranging from nine to twelve millimeters in length and which had become differentiated into a whitish-yellow stipe of about eight-tenths millimeter in diameter by two millimeters in length showed very well the above mentioned internal features. By the time the clava has reached this stage of external differentiation, the interior parts have likewise become highly differentiated. The structure of the stipe is somewhat more homogeneous than during the early stages of development due to the fact that the hyphae have become more uniform in size. One can, however, still distinguish hyphae which are broad in diameter intermixed with and partially obscured by hyphae which are smaller in diameter. The periphery of the stipe is covered by a thin layer of loosely interwoven, hyaline hyphae. By far the greatest amount of differentiation is evident in the head. The central portion of the head is composed of the same hyphal types found in the stipe, while the periphery of the head is covered by a dense layer of pigmented, rather closely interwoven hyphae of the same size as the finer ones of the stipe. This layer is very thin near the base of the head where it gradually merges into the peripheral layer of the stipe. It is thicker near the equatorial region and tends to remain of the same thickness on upward over the apex of the head. Between this peripheral layer and the central core of the head an intermediate zone of hyphae is readily distinguishable because of its looser, weft-like structure. All the hyphae composing this zone are at first of the same size as those of the peripheral layer but are hyaline and more loosely woven. The internal structure of the head of this species is therefore in agreement with that of *C. militaris* (Varitchak, **41**) except that no interzonal layer was distinguished in the latter species. A study of younger stages shows that both the pigmented peripheral layer

and the colorless, loosely woven interzonal layer between this and the core had their origin from hyphae of the core. The hyphae composing these three well-defined zones are septate and the cells are, for the most part, uninucleate.

THE ORIGIN AND DEVELOPMENT OF THE ASCOGONIUM AND RELATED STRUCTURES

The ascogonia originate as branches from various hyphae of the interzonal region. In a few instances these structures were found to have originated from hyphae of the peripheral layer and in a like number of cases there was evidence of their origin from hyphae within the periphery of the core. Ascogonia of *C. militaris* originate in the peripheral layer (Varitchak, **41**). The ascogonia, when first distinguishable, consist of short, usually slightly coiled, three to five septate hyphae, of uninucleate or binucleate cells. Unlike the aseptate ascogonia of *C. militaris* whose nucleus encloses a prominent nucleolus, these of *C. agariciformia* are distinguishable from the vegetative hyphae in their stages only by the fact that they are shorter, somewhat thicker, their nuclei stain more densely and they retain the stain more tenaciously than do the vegetative hyphae (PLATE 28, FIG. 1). In some instances, these structures arise in the larger interhyphal spaces of the interzonal region, and usually occur in groups of three to four. They were best seen in longitudinal sections of young clavae nine to twelve millimeters long. A careful study of the young heads throughout their entire length indicated that the first ascogonia arise near the equatorial region. A majority of the ascogonia found were below the equatorial region of the head, and a more or less complete series of developmental stages was to be noted as one examined the sections from the base up to the equatorial region. Thus, while ascogonia were being initiated in the basal portion of the head, sections through the equatorial region, and less markedly those in the apical region of the head, showed ascogenous hyphae beginning to appear in young perithecia. Development at the apex of the head is advanced beyond that at the base and that in the equatorial region is more advanced than either. These differences in degree of development of these regions were of great value in fixing the sequence of stages of development of

perithecia from the ascogonial stage up to the production of young ascogenous hyphae.

As the ascogonial cells increase in size they likewise tend to become more coiled and their cells become multinucleate. The nuclei, during these early stages of ascogonial development, were so small that positive identification of mitotic figures was not possible, yet the position of the nuclei seemed at times to indicate a recent division (PLATE 28, FIG. 2, 3). No antheridium-like structure was found in the vicinity of the ascogonia. Though two ascogonia often lay very close together, there was no evidence of union between them. This observation is in accord with the situation in *C. militaris* (Varitchak, **41**). The possible presence of pores through the cross septa of the individual ascogonia through which nuclear exchange might occur, structures of rather frequent occurrence among apogamous ascomycetes, will be discussed later.

Concurrent with the development described above certain fine, deeply staining hyphae, much resembling vegetative hyphae, become conspicuous by coiling about and interweaving among the ascogonia (PLATE 28, FIG. 4, 5). These complexes are the "pelotons" of Varitchak. It seems entirely possible that some of these interweaving and coiling hyphae originate from the same hyphae which earlier gave rise to ascogonia. It could be quite easily demonstrated that many of these coiling hyphae originated as branches from the surrounding vegetative hyphae. During the early stages of this process the enveloping hyphae form no particular pattern; very soon, however, these smaller hyphae have increased in number to form a small spherical envelope with the ascogonia at the base (PLATE 28, FIG. 5). Still later the young perithecial envelope consists of many loosely compacted hyphal layers. During this development of the wall of the perithecium certain cells of the ascogonia enlarge considerably and their nuclei increase in number. According to Varitchak (**41**) the ascogonia of *C. militaris* during comparable stages are aseptate, multinucleate structures, the bases of which become inflated to form sacs that give rise to the ascogenous hyphae. It is evident from this study of *C. agariciformia* that the ascogonia are septate structures and that not all the cells give rise to ascogenous hyphae.

Another point of difference between the early stages of perithecial formation in *C. militaris* and *C. agariciformia* is in the manner in which the interweaving vegetative hyphae separate the various enlarged cells of two or more ascogonia which are developing close together. This seemed to indicate that two or more perithecia might arise from a single group of ascogonia. This was found, however, to be the case only very rarely. As a rule, these separating partitions of vegetative were later pushed aside by the developing ascogonia so that only one perithecial cavity resulted. In a few cases the cavities of two perithecia were observed to be confluent during the ascogenous hyphae stage; still more rarely the cavities of mature perithecia were only incompletely separated

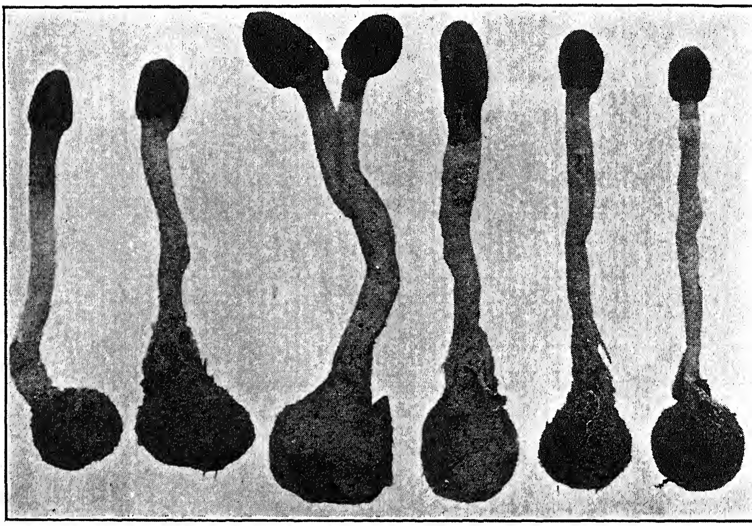

Fig. 1. Photograph of mature clavae attached to the host.

one from the other. These may either represent cases in which the separating hyphal partitions were not pushed aside until after they had been supplemented by others to form a distinct wall, or cases in which two perithecial primordia arose in close proximity to each other and in which, after their walls came in contact, these were displaced in part so that the cavities became partially confluent. Since the perithecia arise from a group of ascogonia, they may possibly prove to be always compound in origin.

DEVELOPMENT OF THE PERITHECIUM

One of the more striking features in the early stages of the perithecium is the development of its wall. Concurrent with the branching of the large cells of the ascogonia to form the primary ascogenous hyphae, to be discussed later, the spherical envelopes of loosely organized hyphal layers surrounding the ascogonia assume a definite polarity (PLATE 28, FIG. 6, 7). As the hyphae of this envelope increase in number the perithecial boundary opposite the ascogonia begins to elongate radially, i.e., toward the periphery of the head. For the sake of clarity in future discussion, this elongating side of the perithecium may be designated the ostiolar end, since the ostiolum will ultimately open there. By continued multiplication and growth of the hyphae of the walls, some of which may have originated from the ascogonia (Varitchak, 41) and grown up to form the inner wall layer of the perithecium, a tension is exerted on the hyphae of the ostiolar end. The hyphae of this region whose tips had heretofore overlapped are progressively pulled apart (PLATE 28, FIG. 9, 12). Elongation of some hyphae keeps pace with the separation of others so that during the stages in question the developing ostiolum is always closed at its distal end. The hyphae which are pulled apart, together with other short hyphae which arose by the branching of those making up the innermost layer of the wall, fall back into the developing cavity. In such a stage the perithecial cavity is rather completely lined by hyphae with free tips.

Concurrent with the appearance of polarity in the perithecium, the large, multinucleate cells of the ascogonia begin to branch variously (PLATE 28, FIG. 6, 7), nuclei migrate fortuitously out into the branches and when these become septate, more than two nuclei are often found in some of the cells. These branches, the primary ascogenous hyphae, grow into the developing perithecial cavity. At this stage hyphae which have their origin in the wall layers are seen to have grown among the primary ascogenous hyphae and ultimately to have formed a partition of vegetative hyphae between these and their bases in the ascogonia (PLATE 28, FIG. 7). Finally, the old, more or less completely emptied ascogonial cells are almost completely hidden by the dense growth

of hyphae between and about them. This is contrary to the situation in *C. militaris* where the ascogonia tend to disappear very early (Varitchak, **41**). In other cases, perhaps just as typical, the ascogonial remains lie at the base, just beneath the floor of the perithecial cavity. At the level of the floor of the perithecial cavity some of the primary ascogenous hyphae multiply by branching and spread out along the floor of the perithecium, ultimately forming a layer of plectenchyma whose cells contain two to four nuclei (PLATE 28, FIG. 9, 13). These cells give rise to the true ascogenous hyphae, but due to their compactness and the presence of other hyphae it is impossible to state how many times a given ascogenous hypha branches before it gives rise to asci.

It is in sections of heads of about this stage that the cells of certain ascogonia are seen to be almost or quite emptied of protoplasmic contents. In certain favorably oriented sections of ascogonial cells, ruptures were noted through septa separating certain of these cells (PLATE 28, FIG. 10). An interpretation of these ruptures is difficult. The evidence in hand at present does not suggest any such sexual relation as has been described for *Polystigma rubrum* (Nienburg, **34**), *Epichloe typhina* (Vincens, **45**), *E. Bambusae* (Gaumann, **17**), *Ophiobolus graminis* (Jones, **24**), *Rhizina undulata* (Fitzpatrick, **16**), or *Ascobolus citrinus* (Schweizer, **37**). These ruptures were not observed in the younger ascogonia though they were sought for diligently. Their occurrence is due, in all probability, to mechanical injury by the closely investing hyphae, or else is the result of some tension caused by the growth of the parts about them. Even though no actual mitotic figures were seen, the multinucleate cells of the young ascogonia apparently result from ordinary mitotic divisions of the one or two nuclei originally present in each. Hence all the nuclei of a given ascogonial cell are believed to be daughter nuclei of the original nucleus or nuclei, rather than derived by the division of nuclei which have migrated from other cells of the ascogonium. This interpretation is in accord with that published for *C. militaris* (Varitchak, **41**).

Even while the perithecia are in this early stage of development the hyphae in the interzonal region show the effects of crowding and stretching. As the perithecia continue their development,

these interzonal hyphae became progressively more disorganized, until finally at maturity the entire layer of perithecia together with the peripheral zone may be peeled away from the central core quite readily.

DEVELOPMENT AND CYTOLOGY OF THE ASCUS

This critical phase in the development of the fungus merits especial consideration since it has received more attention by earlier investigators than have the phases thus far discussed. As has been stated earlier, the asci grow out from ascogenous hyphae which have in turn arisen from the plectenchymatous floor of the perithecial cavity. In some cases the asci arise from typical croziers (PLATE 28, FIG. 14), but intermediate conditions have been noted between these and cases where the asci arise as direct outgrowths of binucleate cells of the ascogenous hyphae (PLATE 28, FIG. 13). When this latter type of origin is frequent along a given ascogenous hypha the picture is like that figured; but probably wrongly interpreted, for *Epichloe typhina* (Vincens, **45**). Proliferation of croziers, as reported for *C. militaris*, were not observed nor were the ascogenous hyphae found to lose their contents and become amorphous as the asci matured (Varitchak, **41**). In all cases the ascogenous hyphae and young asci are extremely small, which, combined with the fact that these structures are compactly arranged in the perithecium, makes it difficult to identify all the variations present.

Regardless of how it originates, the young ascus is always binucleate (PLATE 28, FIG. 15). These two nuclei are indistinguishable. Both are very small and stain homogeneously. None were seen which, prior to fusion, showed a hyalosphere nor could a centrosome be satisfactorily demonstrated such as was described in *C. militaris* (Varitchak, **41**). These two nuclei approach each other and fuse before the young ascus has enlarged to twice its original length. The resulting primary ascus nucleus undergoes a considerable number of changes prior to its first division, all the details of which have not been heretofore published for any species of *Cordyceps*.

One of the more significant of these changes in the primary ascus nucleus is its enormous increase in size (PLATE 28, FIG.

16–26). Contrary to the situation in *C. militaris* (Varitchak, **41**), the nucleus loses its homogeniety very early in its development and one can then readily distinguish the nuclear membrane, nuclear sap, reticulum and a relatively very large nucleolus. Certain sections show a densely staining granule in contact with the periphery of the nuclear membrane (PLATE 28, FIG. 19). Similar and like staining granules have been called centrosomes (Bagchee, **3**; Varitchak, **41**). It could not be definitely ascertained whether this granule is extranuclear or intranuclear in origin. During the early stages of nuclear enlargement the immense nucleolus is encased by the reticulum, though it appears in all cases to be situated near the periphery of the nucleus (PLATE 28, FIG. 16). Later, as the nucleus prepares itself for division, the reticulum becomes organized into a definite spireme. As this structure becomes more thread-like it tends to pull itself away from the nucleolus to the opposite side of the nucleus and is connected with the nucleolus by only a thread or two. At about this stage the nuclear membrane becomes indistinct. During the development of the spireme the nucleolus is somewhat smaller in size, and at the same time very small, densely staining, chromatin-like granules can be observed in the equatorial region of the nucleus (PLATE 28, FIG. 25). Some of the spireme thread are distinct even during such a stage as this. Although the fate of the nucleolus could not be followed so completely as it was in *Pustularia bolarioides* Ramsb. by Bagschee (**3**), the evidence in hand indicates that this body undergoes fragmentation just prior to and during the early stages of the first division of the nucleus. During the period of its growth and reorganization prior to its first division the nucleus is located centrally in the ascus and occupies practically the entire width of it.

The stages of nuclear division seen most clearly during this study were metaphases or anaphases; only rarely was a telophase or intermediate phases evident (PLATE 29, FIG. 27a, 27, 29, 31). Compared with those of subsequent divisions the spindle of the primary division is quite long (PLATE 29, FIG. 27a). Though little claim to certainty is made, because of their small size, four chromosomes were counted in preparations which showed particularly good metaphase and early anaphase conditions (PLATE 29, FIG.

27a). It seems from this and the counts which will be given later for subsequent divisions that the somatic chromosome number for *C. agariciformia* is two Varitchak (**41**) states that two chromosomes were counted during the first division of the fusion nucleus, and later states definitely that the somatic chromosome number for *C. militaris* is two. During this first division, as likewise for the two succeeding divisions, a centrosome is apparent at each pole and is discoid to cap-shaped in form. The concave side of the centrosome is constantly adjacent to the spindle. Such a situation has been described by Jones (**24**) for *Ophiobolus graminis* Sacc. The elongated spindle is situated about centrally in the ascus and describes a wide arc, though it is not uncommonly an elongated S-shaped structure.

The two nuclei which reorganize from this division have a structure similar to the parent nucleus, differing from it principally in their smaller size and the absence of the large nucleolus; this structure being represented by two small, deeply staining, granules (PLATE 29, FIG. 28). In this respect these observations differ from those of Varitchak (**41**) who figures the interphase nuclei of *C. militaris* as homogeneous structures which lack both a reticulum and centrosomes. Judging from their size, internal structure and the infrequency with which these nuclei are found the second division must follow the first immediately. During all the nuclear divisions, except possibly the third, the cytoplasm is rather uniformly dense, though conspicuous vacuoles are present near the apex of the ascus.

The second division in the ascus is, as in other ascomycetes, a simultaneous division of the first two daughter nuclei. The form of the spindle differs little from that of the first division except in its somewhat smaller size (PLATE 29, FIG 29). During the late metaphase of this division four small chromosomes are apparent near the equatorial plate, and other figures of the early anaphase show two such deeply staining granules in transit to each pole. Evidence of astral rays extending from the centrosomes was seen in a few preparations, only. The definite, deeply staining spindle in most cases appears as a single line, though a truer picture of the spindle was sometimes seen. The spindle is so narrow that it is very difficult to demonstrate its individual

fibers (PLATE 29, FIG. 29). In fact, such fine cytological details are seen only when the differentiation of the stain is halted just at the right point during the process of destaining. This difficulty appears to be the rule rather than the exception in work with nuclei so small (Jones, **23**, **24**; Varitchak, **41**). The present writer was able to solve this difficulty in part, and at the same time insure entire asci for the study of later cytological phenomena, by making use of smears of asci which were stained and studied in toto.

The four nuclei which reorganize from this second division are similar to the parent nuclei except for their smaller size (PLATE 29, FIG. 30). Although there appears to be a longer interval between the second and third divisions than between the first and second, the nuclei do not increase in size appreciably nor reorganize as resting nuclei. The third division is likewise simultaneous and the spindles are predominantly parallel to or but slightly oblique to the long axis of the ascus (PLATE 29, FIG. 31, 32). A similar orientation of these spindles was found in *C. militaris* (Varitchak, **41**). Such an orientation of spindles results, of course, in a linear arrangement of the reorganizing nuclei. This arrangement seems particularly significant in connection with the form of the spores later to be organized about these eight nuclei.

The matter of how the filamentous, multiseptate, ascospores so characteristic of this genus are developed from the product of the third successive division of the fusion nucleus in the ascus has been the subject of diverse opinions (Lewton-Brain **29**; Faull, **14**; Maire, **30**; Varitchak, **41**). As Faull (**14**) has remarked, Lewton-Brain attempted a cytological investigation on *Cordyceps ophioglossoides* with material that was not properly fixed. In consequence he probably mistook nucleoli for nuclei. Although he figures no nuclear divisions, Lewton-Brain states that there is no evidence of spore delimitation in the ascus until after a great number of nuclei are present in it. Later, he says, these nuclei arrange themselves into eight rows and the cytoplasm about each row becomes delimited by longitudinal cleavages to form eight, multinucleate, aseptate spores; each of the spores becoming septate only after additional nuclear divisions. Faull (**14**) saw uni-

nucleate spores of the same species and thinks that the multinucleate spore probably arises from the uninucleate one by nuclear divisions accompanied by septation. Maire (**30**) found uninucleate spores in *C. agariciformia* but did not follow their development. Varitchak (**41**) in his work on *C. militaris* is in agreement with Faull.

The present writer finds that following the third division the cytoplasm about the eight reorganized nuclei cleaves to form an ascospore initial. No actual cleavage planes are noticeable until after the eight ascospore initials have completely reorganized, but even during the course of the third division the cytoplasm becomes particularly dense near the poles of the spindles. This condensation of cytoplasm continues until one can readily distinguish the symmetry of the young ascospore initials, even before cleavage planes are evident. A very careful study of the process of differentiation of the ascospore initials has failed to demonstrate that astral rays in any way initiate the cleavage of the cytoplasm during these stages. The spore initials seem rather to be first delimited by condensation of the cytoplasm about the nuclei followed by a series of small vacuoles which form and coalesce along the periphery of the spore initials, thus cleaving the cytoplasm. Later, after cytoplasmic cleavage has progressed considerably, astral-like rays can be demonstrated, in some preparations, emanating from a densely stained area at one end of the spore initial (PLATE 29, FIG. 37).

Thus from the evidence available, the mode of formation of the ascospore initials seems to accord rather with that described by Faull (**14**) and Jones (**23**, **24**) than with that described by Harper (**22**) and Varitchak (**41**). It should be noted, however, that the ascospore initials in *Cordyceps militaris* are delimited by astral rays, according to Varitchak.

The ascospore initials when differentiated, lie somewhat obliquely to the long axis of the ascus and tend to occupy the entire width of the ascus (PLATE 29, FIG. 34–36). Judging from the staining reactions of the boundaries of these structures as compared with the surrounding epiplasm—and later, as compared with the staining reactions of the boundaries of somewhat older stages

of these structures—no spore wall is formed about the spore initial, merely a cytoplasmic membrane (PLATE 29, FIG. 34).

As in *C. militaris* (Varitchak, 41) the growth of these unicellular spore initials must take place extremely rapidly, for very few stages in their growth were found. In practically all stages seen, even the youngest, the nuclei were actively elongating indicating that the uninucleate spore initial stage is of short duration (PLATE 29, FIG. 34–39). In those stages found there was no appearance of cross septa until after the spore initial had grown considerably in length. Such a condition was described for *C. militaris,* but the suggestion was made that possibly the newly formed septa were difficult to stain. This explanation might suffice were it not that later cross septa are clearly evident, although the spores are elongating and nuclear divisions are still in progress. During the early growth of the spores the nuclei elongate greatly in such a manner as was described for *Rhytisma acerinum* (Jones, 23). Just how they become organized again is not clear (PLATE 29, FIG. 39). Later the nuclei divide mitotically but the figures are too small to show any detail except at anaphase (PLATE 29, FIG. 40). At this stage the small deeply stained spindles, with a mass of chromatin at each pole, are quite conspicuous. Even after tranverse septa become apparent the spores continue to grow in length, each cell of the spore apparently being capable of independent elongation and division, though the terminal cells seemingly become inactive before the median ones do. The young spores are always eight in number and at maturity are thirty-five to fifty septate; each cell being uninucleate. Prior to being released from the ascus many spores break up into unicellular segments.

PECULIARITIES OF THE ASCUS

The development of the capitulum of the ascus first becomes noticeable as the primary ascus nucleus is preparing for its first division (PLATE 28, FIG. 19, 20). Such a stage shows the apex of the ascus somewhat enlarged and practically void of the granular contents so characteristic of the remainder of the ascus. Further differentiation proceeds rapidly so that by the time the three successive divisions of the nucleus have been accomplished the apical

portion of the ascus wall has become considerably thickened and a pore or core of clear cytoplasm is seen to extend up into the thickened area of the wall (plate 29, fig. 33). At maturity, the apex of the ascus is covered by a much thickened, conical, lid-like process which is penetrated centrally by the narrow pore or ascostome. During the period in which the three successive primary nuclear divisions are accomplished the ascus has been increasing steadily in length, so that by the time the capitulum is completely formed the ascus has about reached its definitive length.

Another peculiar feature of the ascus, aside from certain deformities which perhaps never reach maturity or else become normal before they reach maturity, is due to the presence of crystals in it. These structures are quite evident in fresh material, and their effect is quite noticeable in sections prior to being stained. Apparently the various treatments through which the material was passed (fixation, dehydration, etc.) dissolved the crystals, but the distortion of walls and cytoplasm due to their presence were preserved (plate 29, fig. 42). These crystals are noticeable, even in young asci, but as the asci approach maturity the distortion caused by the structures is quite pronounced. Often the ascus walls and even the spores are bulged to such an extent that rupture seems imminent. It is suggested that these crystals may play a rôle in liberation of the ascospores.

THE MATURE CLAVA AND PERITHECIUM

During the course of ascospore development, as described above, the clava has been steadily increasing in size. The length of the stipe seems to depend on the depth of the parasitized host under the soil, while the dimensions of the head are probably related to nutritional factors. The stipe is usually white to grayish-white in color while the head is greenish-black when the clava is mature (text fig. 1). Such mature heads, when placed under humid conditions, soon become covered by a hyaline, viscous mass of spore segments and almost complete spores. These spore segments swell considerably and often germinate while still in contact with the clava. On nutrient medium these germinating spore segments form abundant conidia resembling the form genus *Spicaria*.

The mature perithecium is ovate to pear-shaped in form, its broad base resting in the much weakened and distorted interzonal area and its long neck protruding through the pigmented peripheral layer to the outside. So evenly do the hyphae of the neck fit into those of the peripheral layer that, as suggested by Lewton-Brain (29), the perithecia appear on superficial examination with low magnifications as invaginations of the peripheral layer. At maturity the perithecial cavity is completely filled with asci in all stages of development. The paraphyses-like hyphae together with the hyphae which were dislocated during ostiolar development have gelatinized and disappeared for the most part, in all probability being used as food by the developing asci or else as an osmotic fluid during the enlargement of the perithecium. At the base of the perithecium may be found the asci which have emptied and collapsed, thus making room for a new crop of asci originating from the still active ascogenous hyphae. It is not know how long a perithecium continues to produce spores, but it is conceivable that the active ascogenous hyphae may continue to produce new asci and spores for weeks. The exterior walls of the mature perithecium, at its base, are very nearly in contact with those of the adjacent perithecia. The walls have, throughout, lost their plectenchymatous structure, being now pseudoparenchymatous. The ostiolum, which is now continuous and open from the perithecial cavity to the outside, is lined with deeply staining periphyses which have arisen from the walls of the ostiolum. Scattered among the asci and often in the ostiolum (they were for the most part washed away) are many complete spores, but for the most part the spores have broken at the cross septa to form spore segments.

SUMMARY

1. This investigation consists of a study of the development of *Cordyceps agariciformia* (Bolt.) Seaver from the initiation of the clava until the spores mature.

2. This species begins its parasitism as an interhyphal parasite, but soon invades the hyphae of its host, *Elaphomyces*, and becomes an intracellular parasite.

3. Apparently the clava is formed after the parasite has com-

pleted the absorption of food from its host, and is initiated by hyphae which have arisen from the germination of pseudo-sclerotial cells lying just beneath the cortex of the host.

4. The young clava consists of a mass of hyphae, the central ones being somewhat larger in diameter and somewhat more compacted than the peripheral ones. Very early, however, the stipe becomes differentiated externally into a stipe and head.

5. Internally, the stipe consists of (1) a central core of compact, hyaline hyphae of two sizes which run predominantly parallel to the long axis of the clava and (2) a peripheral layer of fine, hyaline, loosely interwoven hyphae. The head consists of (1) a central core, which structurally is a continuation of the core of the stipe, (2) a peripheral layer of fine, pigmented and intricately interwoven hpyhae, and (3) an interzonal region of hyaline, loosely woven hyphae, of the same diameter as those of the peripheral layer.

6. The ascogonia arise predominantly within the interzonal region in groups of three to four as somewhat enlarged hyphae, each three to five celled. Certain cells of these structures rapidly enlarge and become multinucleate. No evidence of an antheridial structure was seen.

7. Fine, densely staining hyphae coil about and enclose the ascogonia and thus initiate the formation of perithecia.

8. The perithecial primordium is, during its earlier stages, more or less globular but soon the enveloping hyphae assume a polarity of growth and the true wall and ostiolum are formed.

9. Later the multinucleate cells of the ascogonium branch to form primary ascogenous hyphae. These hyphae elongate to form a plectenchymatous layer of binucleate cells along the floor of the perithecial cavity, which in turn give rise to the true ascogenous hyphae.

10. The ascus arises either from a crozier or as a direct outgrowth of a binucleate cell of an ascogenous hypha.

11. The cytology of the ascus and spores has been studied in considerable detail. The ascospore initials arise by vacuolar cleavage rather through the activity of astral rays. These structures are at first unicellular and uninucleate, but very soon elongate to form the mature, multiseptate spore. A septum is not formed

until after the spore initial has attained considerable length. Even after septa begin to form, the spores continue to increase in length by means of independent elongation and division of its cells, until at maturity the spore is thirty-five to fifty septate.

12. The somatic number of chromosomes for this species is two.

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EXPERIMENT, GEORGIA.

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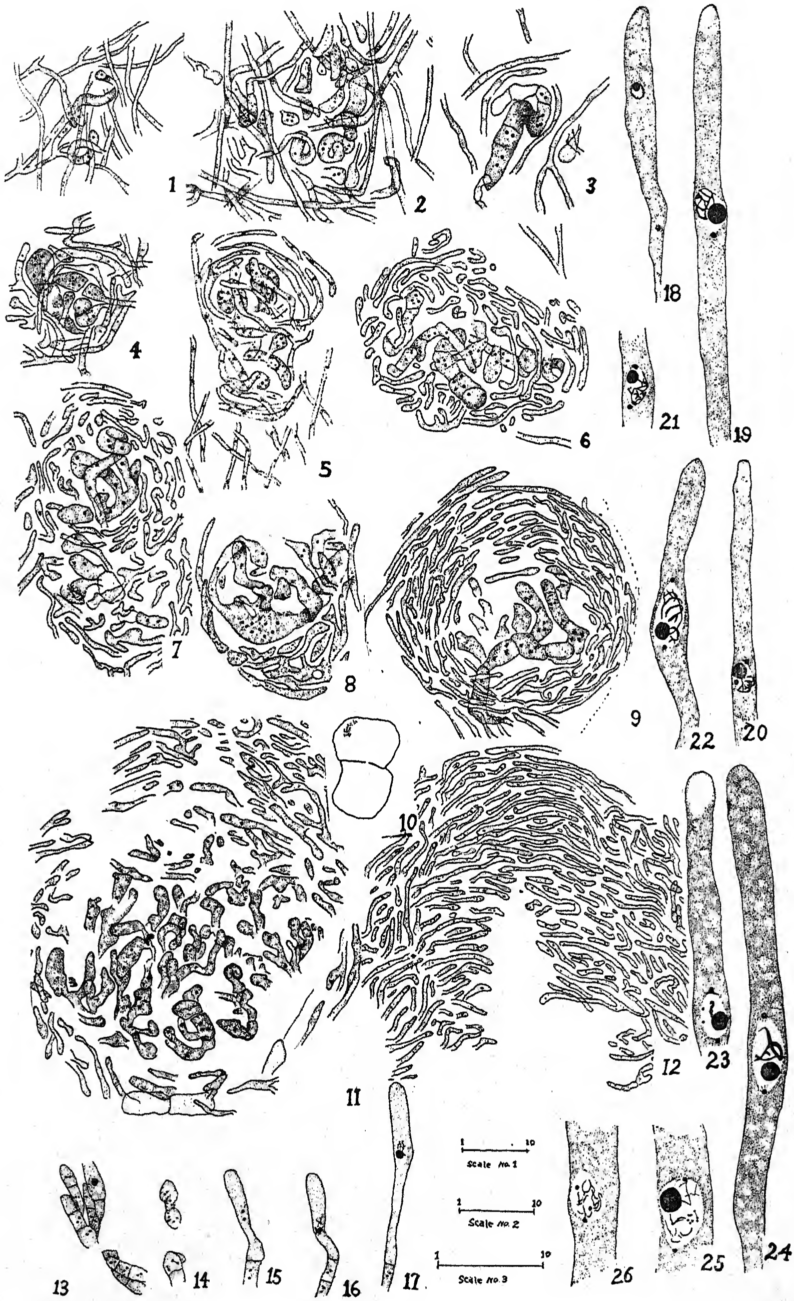
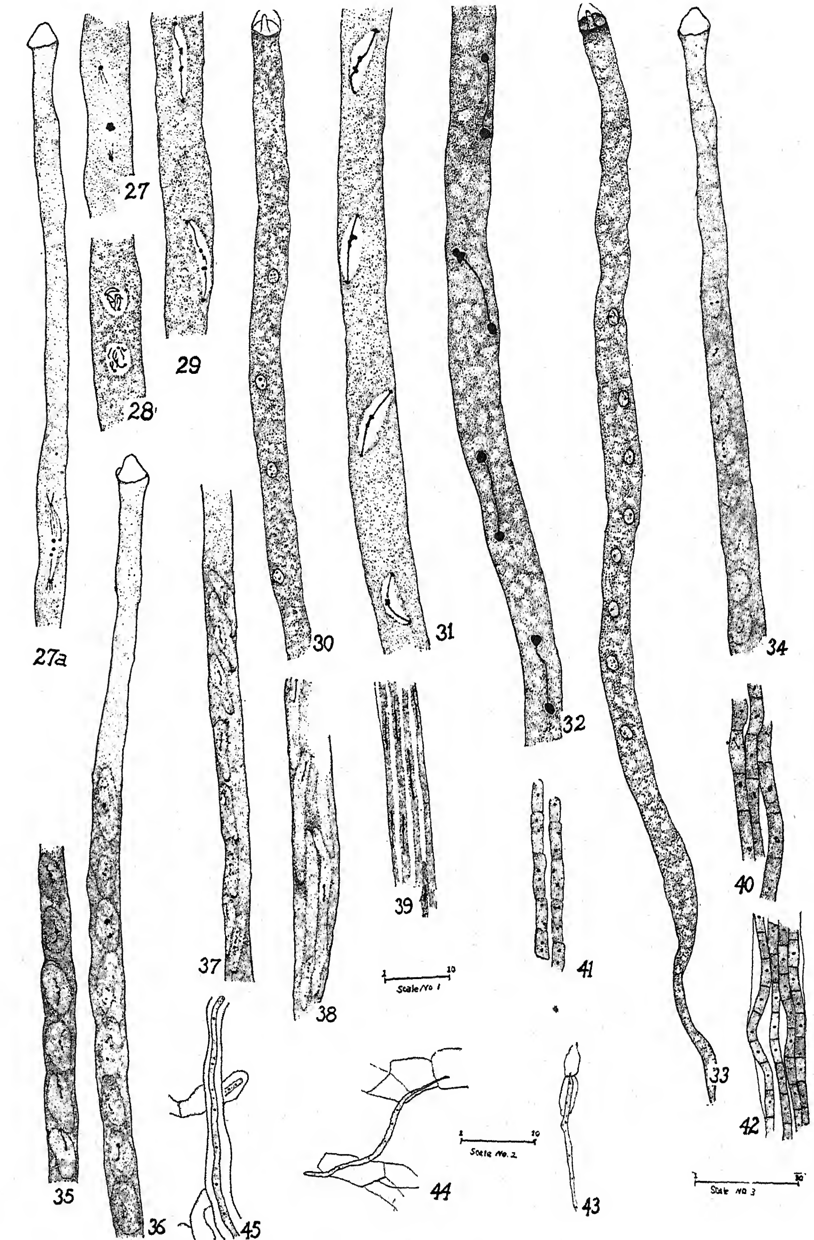
EXPLANATION OF PLATES

PLATE 28

All figures drawn with the aid of an Abbe camera lucida (Zeiss). Scale 1 Zeiss 3 mm. Apochr. immersion lens (N. A. I. 40) with Zeiss 20 × comp. ocular. Scale 2 Leitz 1/12 immersion lens (N. A. I. 30) with Leitz 15 × periplan ocular. Fig. 1, 4, 5, 8, 10, 11, 12 drawn to Scale 1, others to Scale 2. Fig. 1, A group of young ascogonia which are slightly larger and stain more deeply than the surrounding vegetative hyphae; 2, Somewhat older ascogonia, showing a distinct coiling and enlargement; 3, A single ascogonium showing enlargement of certain of its cells; 4, Ascogonia in which certain cells have become multinucleate. Note enveloping hyphae; 5, Portions of ascogonia whose tips overlie each other, though there is no evidence of connections between them; 6, A group of ascogonia showing certain cells that are beginning to branch; 7, A more advanced stage than the preceding showing a few of the partition hyphae about the equatorial region; 8, Portion of an ascogonium showing manner of branching of much enlarged, multinucleate, ascogonial cells; 9, An obliquely cut section showing a few primary ascogenous hyphae in the young perithecial cavity; 10, Two, much enlarged, empty, ascogonial cells taken from the base of a young perithecium bearing ascogenous hyphae. A rupture in the septum is evident. 11, Semi-diagrammatic sketch of the interior of a young perithecium. The variety



CORDYCEPS AGARICIFORMIA



CORDYCEPS AGARICIFORMIA

in structure of the ascogenous hyphae is striking. 12, Apex of a young perithecium showing the mode of formation of perithecial wall and ostiolum. Fig. 13, 14, 15, 16, 17, 20, 21, drawn to Scale 1; Fig. 18, 23, drawn to Scale 2; others to Scale 3. Fig. 13, Portion of the plectenchyma of the floor of a young perithecium, showing asci arising directly from binucleate ascogenous hyphae; 14, Two views of croziers found at the base of a young perithecium; 15, A young, binucleate ascus; 16–26, A series showing the growth of the ascus and progress of nuclear changes prior to the first division. The polar granules present in each case are interpreted as centrosomes. Fig. 19, 20, 23, beginning of operculum formation.

PLATE 29

All figures drawn with the aid of an Abbe camera lucida (Zeiss). Scales as in Plate *28*. Fig. 27a, 30, 34–45, drawn to Scale 1, others drawn to Scale 3. Fig. 27a and 27, First division of the primary ascus nucleus at early anaphase and metaphase respectively. 28, A binucleate ascus, the nuclei preparing for the second division; 29, Division figure of the second mitosis. Due to their small size, the behavior of the chromosomes at metaphase could not be ascertained. 30, A four nucleate ascus, the nuclei preparing for the third successive division; 31–32, Division of the third nuclear division, fig. 31 showing chromosomes, fig. 32 showing a late anaphase condition; 33, An eight nucleate ascus; 34–42, The organization of the spore initials and the growth of these to mature spores in order of sequence. Fig. 34, In preparations of this stage the activity of astral rays could not be detected. Fig. 35, The nucleus is shown elongating to the periphery of the spore initial. Fig. 37, Shows the crescent-shaped centrosome-like body at the basal pole of the elongating spore initial. Fig. 39, Elongated nuclei in young spores. Fig. 40–41, Nuclear division in cells of young spores. Fig. 42, Distortion of spores and ascus wall caused by crystals; Fig. 43–45, Illustrating certain phases of the host relations. Fig. 43, An intracellular hypha has pushed an attenuated tip into the adjoining host cell. Fig. 44–45, Intracellular hyphae of the parasite.

FURTHER TESTS FOR HORMONE ACTION IN NEUROSPORA

Alice Aronescu

(with plate 30)

The recent note in Science by Plumb and Durell (**1933**) reporting the effect of the female hormone theelin on preventing or delaying the formation of zygospores by *Rhizopus nigricans* reminds us again that some such means may be a valuable aid in determining whether the mating of two strains of a heterothallic fungus is a matter of *sexual* reproduction. This is of especial interest in view of the unusual results previously reported by Moreau-Moruzi (**1931**) on experiments dealing with the nature of the stimulant which induces the formation of perithecia with asci in the heterothallic species *Neurospora sitophila*. Two strains of opposite mating reaction were cultivated, one in each arm of U-tube cultures. The authors state that perithecia formed on one of the agar surfaces, as a result of diffusion of something analogous to hormones from one of the mycelia to the other some distance away. These perithecia matured without intimate contact between the two mycelia and without an exchange of nuclei or any act of copulation. Later (**1932**) they inoculated a petri dish culture, for example, on one side with strain 17, and on the other side with a strain of opposite reaction. They did not obtain perithecia along the line where the two mycelia came into contact, but they were formed on either side of this region and at a certain distance away from it. Their interpretation is the same as before, namely, perithecia were formed as the result of an action taking place between the two strains at a distance.

Experiments along this line were undertaken by Dodge (**1931**) who repeated in substance the work done by the French authors. He was unable to find any evidence of hormone action. In each case where perithecia were formed, they were produced, he says, as a result of the coming into contact of the two strains of opposite sex reaction.

The writer (1933) continued the experiments trying to determine through another method, what it is that induces the formation of mature perithecia with asci. In interpreting the results, we relied on two important facts, found by Dodge: (*a*) the mendelian segregation of the factors for conidial and sex characters (1930); (*b*) the regularity with which perithecia are obtained in every case in which one spermatizes or conidiates incipient perithecia, "sclerotia," of one strain with spermatia or conidia of the opposite "sex" (1932, 1933) showing that between the strains there is an actual exchange and fusion of nuclei.

In a recent paper (1933) the writer has shown that if we assume the perithecia in U-tubes matured as a result of the action of diffusible hormones, the results obtained after germinating the eight spores of an ascus should be entirely different from those obtained if a fusion of nuclei derived from the two strains employed had taken place. No case has yet been found in which such hormones transmit and imprint hereditary factors. One suspects that their action is, at most, of secondary importance, an action which accompanies the principal act of copulation. In this case, the eight spores of an ascus from a perithecium obtained in the manner described by Moreau-Moruzi, should give, after germination, eight strains absolutely like the particular strain cultivated in the arm where the perithecia finally appeared.

In the paper mentioned above we reported having analyzed the perithecia formed in the connecting arms of eight U-tubes, where two mycelia, one albinistic and the other conidial, of opposite reaction, were grown. From an analysis of over fifty asci from different perithecia, we found that in each case the segregation for conidial and "sex" factors had occurred in a perfectly normal mendelian manner, as would have been expected, considering that a fusion of nuclei from the two mycelia that formed these perithecia had taken place.

The only objection that could be made against the experiments reported was that strains exactly identical to those employed by Moreau and Moruzi in France had not been used. They used a strain referred to as "Souche de Bordeaux" found by one of the authors (Moruzi, 1932) on mushrooms. With this strain they grew a strain of American origin. Considering it possible that

the Bordeaux strain, while it might be identical, morphologically, with the American strain, yet might, nevertheless, behave differently physiologically, we asked Professor Moreau to send us this particular strain. This he was very glad to do, and through his courtesy new experiments were possible. The results obtained are here reported. In order to obviate any confusion it should be noted, however, that the Bordeaux strain when crossed with our A and B tester strains proved to be of "sex" A and not of "sex" B as indicated in all the publications by Moreau and Moruzi.

Three series of cultures in U-tubes were made in December, February and April. In all cases we inoculated one arm of the U-tube with the Bordeaux strain and the other arm with some one of the four "sex" B strains from ascus 56,[1] namely 56.1, 56.2 (non-conidial), 56.3 and 56.4 (conidial). Each combination of cultures was twice repeated in each series, so that every series consisted of eight U-tubes.

We muse state, in passing, that the way in which the growth of the mycelia develops towards the connecting arm, as well as the drying out of the agar and the appearance of air pockets (Dodge, 1931) depend to a large extent upon the way in which the medium is prepared. A small variation in the concentration of the medium, or a slightly prolonged sterilization may bring about a hastening of drying out of the agar in the two arms, which gives different and very interesting aspects to the developments in the tubes.

The U-tubes of the first series were inoculated on December 12, 1932. With a magnifying glass we were able to follow in each case the growth of the two mycelia towards the connecting arm and mark their progress with a colored pencil each day. All of the tubes presented, in general, the same aspects. By December 19, that is, at the end of not more than seven days, the pairs of mycelia in each U-tube had come together in the connecting arm. The drying out of the agar started very soon after the inoculation, so that in the interval from December 19 to December 29 the air pockets completely united in the middle arm, provided the oxygen for the formation of perithecia in this region. In two cases the

[1] See Mycologia 22: 1930 for full account of cultures from ascus no. 56.

perithecia advanced from the middle of the connecting tube towards one of the arms and in one case they even reached to the surface of the agar in the arm with the 56.2 strain (PLATE 30). In not a single instance were perithecia obtained first on the agar surface or before the two mycelia came in contact with each other.

As compared with Dodge's experiments, the advance of the mycelium, down through the agar, the formation of the air pockets, and the formation of perithecia was accomplished in a very short interval of time. It could be claimed that, because of this rapid development, the hormones did not have time enough to diffuse through the agar and thus induce the formation of perithecia at a distance. In order to prevent this drying out, two other series of experiments were performed. In one case we prepared a series of tubes in a way to avoid as far as possible accidental contamination. In the case of the conidial strains we started from single spore (conidium) cultures. In order to prevent drying, the tubes were placed under a bell jar which was kept moist with water-soaked filter paper placed on the inside. The bell jar as well as the glass plate on which the basket with the U-tubes was placed were disinfected with either alcohol or bichloride of mercury every time the jar was removed to observe the progress of the mycelia. The cotton plugs were thoroughly sprayed with alcohol as an additional precaution.

In six of the tubes the mycelia came very close together in from five to seven days. In two other tubes they remained separated at an appreciable distance. Only after one full month did one of the tubes begin to dry out. The remainder of the tubes did not begin to form air pockets until two or three months after the inoculation. The drying continued at a slow pace, the joining of the air pockets varying with each tube. After from five to seven days from the time the air pockets came together in the connecting arm, perithecia appeared near the point of contact of the two mycelia, and progressed thereafter toward each of the two arms. In two of the tubes the fusion of the air pockets was accomplished only four months after inoculation. Possibly, by this time, the mycelia were too old to start a new growth and no perithecia were formed. In this case as well as in the preceding series of cultures, perithecia did not appear at the surface of the agar but only in the connect-

ing arm and then only after the two opposite strains came into contact in the presence of air.

In the case of the second set of experiments, made for the purpose of preventing the agar from drying out, instead of placing the U-tube under a bell jar, water was introduced into the arm just as soon as the air pockets began to form, which was on the third day after inoculation. Even though precautions were taken in introducing the water, the operation afforded in two cases an opportunity for contamination by the conidia which are very light and float in the air. In two of the tubes a few perithecia developed on the agar surface. The analysis of segregations in seven asci from five different perithecia as well as the fact that perithecia appeared just five days after introducing the water, proved conclusively that the perithecia were matured as the result of accidental contamination. This merely illustrates that one cannot be too careful in culturing *Neurospora,* and it can be said that in all of our other experiments when the plugs were not removed, no perithecia were formed on the surface of the agar in either of the arms of the U-tubes.

We did not always find, like Dodge (1931), that the drying out occurs first in the arm with the conidial strain. The numerous variations obtained led us to believe that it may be largely a matter of chance or that it may depend upon the abundance of mycelia present, be it albinistic or conidial or the relative tightness of the cotton plugs in the opposite arms and other factors.

Denny (1933) has measured the oxygen requirement of *N. sitophila* for the formation of perithecia and has found that, at room temperature, the lowest oxygen concentration at which perithecia formed readily was about 1 to 2 per cent by volume. Reducing the oxygen below 0.5 per cent the formation of perithecia was inhibited.

Recently, we have used methylen blue in our agar medium as an indicator for the amount of oxygen present in the agar. The agar is colored light blue by adding a few drops from a very dilute solution of this substance, before sterilizing. If the inoculated tubes are kept in moist condition so as to avoid immediate drying out of the agar, the fungus, by growing through the medium, consumes oxygen, reduces the methylen blue and by the time the

mycelia arrive in the connecting arm, the agar loses much of its blue color. As soon as the air pockets begin to form, the indicator, by oxidation, gradually regains its blue color and marks therefore, the presence of a new supply of oxygen in the agar. It is also very interesting to note that this substance does not seem to hinder the growth of the fungus and that perithecia develop, in the connecting arm, in the usual manner. This was true even for concentrated solutions.

A last attempt to check the possibility of hormone action as the main factor in the production of mature perithecia, was made with petri dish cultures. Moreau and Moruzi (**1933**) inoculated pairs of strains of the same reaction at two opposite sides of a petri dish. They obtain, among numerous big "sclerotia," also mature perithecia with asci. They maintain that when a culture is inoculated on opposite sides or in two different places with the same strain, the production of large sclerotia such as are never found, they say, in cultures started from a single inoculation, is induced. This stimulation, according to them, goes so far in a few cases as to lead to the formation of mature perithecia. This would mean that each strain has all of the potentialities sexually as well as morphologically, and that heterothallism is merely of secondary importance in securing a greater abundance of perithecia.

We repeated their experiments with a few strains obtained from different sources, namely, Wolf, Wittrock, 56.1, 56.2, 56.3, 56.4, all of which are "sex" B; and also Bordeaux strain, 56.5, 56.6, 56.7, 56.8, which are "sex" A.

All possible combinations were made between strains of the *same* "sex" and each combination was made in triplicate. In order to prevent any possible contamination, we started, in the case of the conidial strains, from single spore cultures. The petri dishes were thereafter kept in an incubator and were sprayed occasionally with bichloride of mercury. During an entire month, the sclerotia that showed a more pronounced development were examined from time to time with a binocular through the upper cover. After this interval, about one hundred of these bodies were crushed, mounted and observed under the microscope, but none of them showed any asci. We have never in any of our cultures derived from single normal spore strains of heterothallic species, obtained perithecia with *asci.*

Our U-tube experiments, repeated in three series, fully convince us that in all cases where perithecia were obtained they were the result of the contact between strains of opposite mating reactions.

Theoretically, in the case of the *Neurospora*, we do not exclude, other necessary conditions being right, the possibility of having perithecia develop *more readily* under the influence of a stimulant such as described by Moreau and Moruzi. Many cases are known in the literature for different fungi when the presence of colonies of bacteria or other entirely different fungi so change the environment as to induce ascocarp formation.

In 1903, Molliard claims that the only way to obtain ascocarps in pure cultures of *Ascobolus furfuraceus* is to inoculate that culture with a bacterium which was first isolated from contaminated cultures of this fungus. Recent work done by Dowding (**1931**) shows that this species is heterothallic and that apothecia can be obtained in perfectly pure cultures if the two opposite strains are grown together. The bacteria might help nevertheless to bring about the right conditions in the medium for the formation of perithecia provided both of the strains were present.

Heald and Pool (**1909**), Dodge (**1912**), Sartory (**1912**, **1916**, **1920**) and McCormick (**1925**) have also shown that the introduction of colonies of certain bacteria or certain species of fungi, frequently induces ascocarp formation in cultures that otherwise would have remained sterile.

Our cultures inoculated with two strains of the same mating reaction show, from time to time, large sclerotia, but never any mature perithecia with asci. These large sterile bodies appear just as frequently in cultures inoculated with only one strain.

If the formation of perithecia in *Neurospora* may sometimes be the result of only a nutrition or a hormone stimulus, and not of a fertilization process ending in fusion between nuclei, the writer is of the opinion that, in such cases, the final proof that the cultures had or had not been contaminated must rest with an analysis of the eight cultures to be obtained from individual asci. This would show in case of hormones or nutritive action alone, all of the cultures to be alike as to sex reaction and conidial characters.

THE NEW YORK BOTANICAL GARDEN.

EXPLANATION OF PLATE 30

Neurospora sitophila

A. U-tube culture (inoculated December 12) with the Bordeaux strain, B. s., growing in the right arm, and strain 56.4 in the left arm. Photograph taken twenty days later. The air pockets had met at the center of the connecting arm a few days previously. Perithecia soon began to develop progressing toward the arm containing strain 56.4.

B. The same type of culture except that the albino non-conidial strain, 56.2, was grown in the left arm of the U-tube. Here, after the air pockets had met, the mycelium of the Bordeaux strain advanced along the wall of the left arm of the tube so that perithecia, starting from the bottom, developed all along up to the agar surface of the 56.2 arm.

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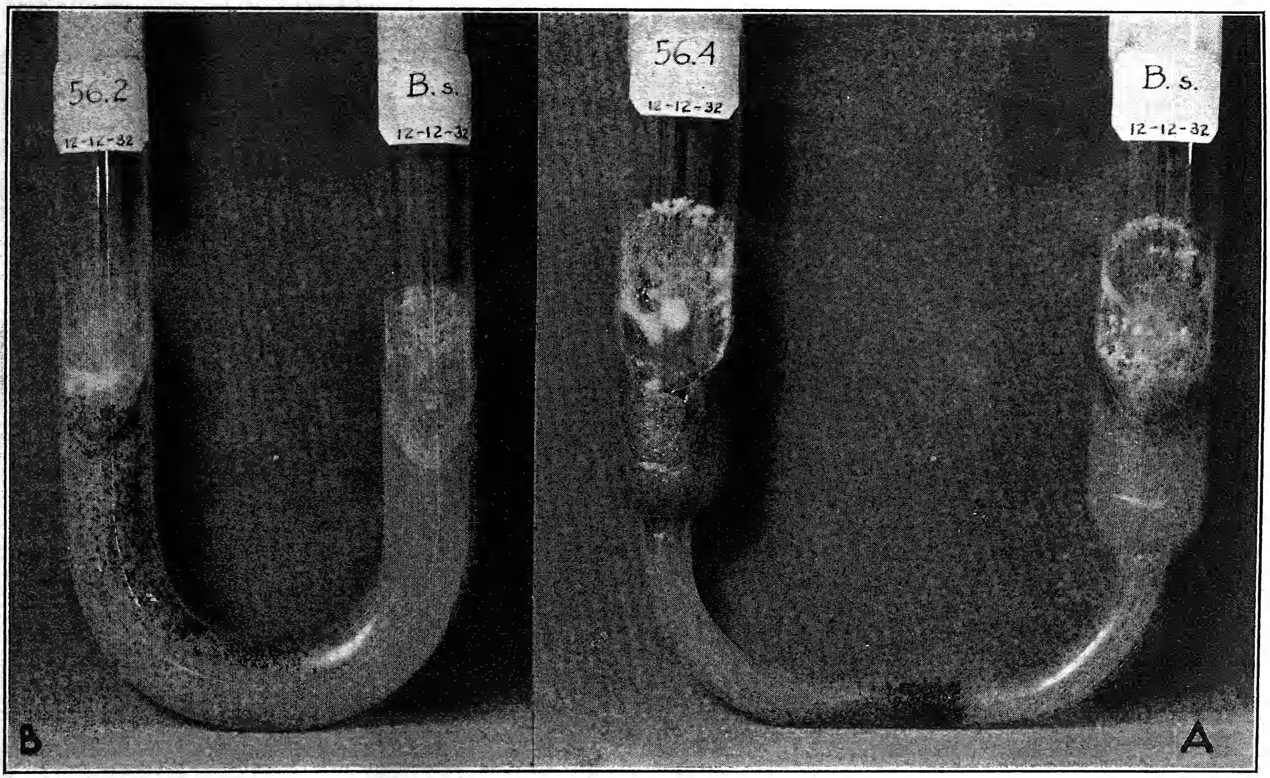


NEUROSPORA SITOPHILA

NEW AND INTERESTING FUNGI

H. C. Beardslee

(with 3 text figures)

During the past year, it has been the good fortune of the writer to collect and study several species of fleshy fungi which seem to be of unusual interest, and the following notes in regard to them have been prepared in the hope that they may be of service to other students of these plants.

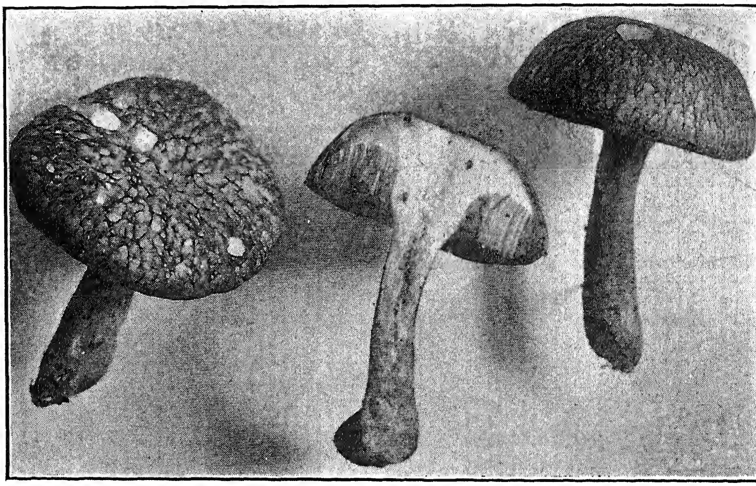
Tylopilus conicus (Rav.) comb. nov.

One of the greatest thrills of the season was the discovery, in Florida, of this long lost species. It was discovered and described in 1853 by Ravenel, who found it in " damp pine woods " in South Carolina, and its characters are so striking that in spite of the fact that it does not seem to have been reported since his day, it is still retained in our literature, Peck in " Boleti of the United States," and Murrill in North American Flora both listing it.

When found it was at once recognized as one of the " pink-spored " Boleti but it was not one of the five species with which I was familiar and my scanty winter library had nothing which dealt with Boleti. A specimen and colored photograph were, however, sent to Mr. L. C. C. Krieger who pronounced it Ravenel's species, and subsequent study seems to confirm this determination.

As will be seen from the figure of our plant, its appearance is very striking. The pileus is covered everywhere with appressed yellow fibers forming a coarse network, whose bright yellow color is very striking against the white surface of the cap. No species of *Boletus* known to me at all resembles it. The tubes are flesh color with their mouths round and .4–.7 mm. in diameter. The stipe is slender 4–5 cm. long and 8–12 mm. in diameter. The

spores were 15–18 by 6–7 mc. A good spore-print was obtained, and was distinctly pink, but rather darker than the spores of some of this group. Their color indicated that the species belongs in *Tylopilus* rather than in *Ceriomyces*.



Tylopilus conicus

Plants were found in two different stations, in both cases in cut-over pine woods. It may prove to be not rare in Florida.

CHAMAEOTA PUSILLA PAT.

The genus *Chamaeota* is of peculiar interest to all mycologists. It can be recognized at sight by its pink spores and its annulate stipe, but its species are so few in number (only about a dozen in all!) and so rare in occurrence that few collectors have ever seen a living specimen. Ricken does not list any species for Germany; Rea gives two for England but has never seen either of them, and only two species have been found in the United States, each reported from one station in Michigan.

Under these conditions it can easily be understood that it was with peculiar pleasure that what seems to be *C. pusilla* Pat. was found near Oviedo, Florida, in December.

Our plant was small, with the pileus 1.5 cm. broad, and lemon yellow streaked with dark brown fibers, which were most abund-

ant at the center. It had a distinct annulus, but one of an unusual character, which does not seem to have been sufficiently emphasized. The basal third of the stipe had a fibrous sheath, which was yellow and apparently continuous with the epidermis of the pileus. The pileus had broken away from this, leaving a distinct ring on the stipe. Both the annulus and the base of the stipe were yellow, while the stipe above the annulus was white. The spores were sub-globose to globose, 5–7 × 5 mc.

As to the identity of our plant it is difficult to speak with finality. Mr. Alexander Smith of the University of Michigan has kindly compared my material with the specimens and figures of the two Michigan species. *C. sphaerospora* Peck seems to be amply distinct from our plant. *C. mammillata* Longyear which was found near Greenfield, Michigan, corresponds well in form and size and in microscopic characters. The pileus is however described as having a "prominent mammiform projection" and as being white with a lemon yellow umbo, and the stipe is not described as sheathed, with a yellow base.

The Florida plant when growing was round-campanulate and obtuse, but in drying it developed a marked umbo. It is also to be noted that while Longyear does not speak of the stipe as being sheathed, his figure indicates a sheath quite distinctly, and since the sheath is continuous with the surface of the pileus, if the pileus is white the annulus and base of the stipe would of necessity be white. It would seem possible that our plant should be considered a form of Longyear's species with the pileus yellow instead of white.

Among the European species *Annularia* (or *Chamaeota*) *Fenzlii* and *A. pusilla* Pat. (Bull. Soc. Myc. Fr. **4**: 24, 1888) which is described as a "miniature *A. Fenzlii*" seem close to our plant. Gillet's figure of the first species shows a plant differing from ours only in size. It has the same yellow color, same rounded pileus, and the same stipe, white above and yellow-sheathed below, but its size is more than twice as large. *A. pusilla*, however, seems very close to our species. The stipe is said to be yellow, instead of white above and yellow at the base, but otherwise both figure and description fit well.

For the present it seems best to consider our plant *C. pusilla*

Pat. It is questionable whether *C. mammillata* Long and *C. pusilla* Pat. would not best be considered forms of *C. Fenzlii* as there is little save color and size to distinguish them, and color and size are notoriously unreliable characters. This time must decide, but in the meantime we have at least a new American station for this rare genus.

PLUTEUS COCCINEUS MASSEE.

Syn. *P. caloceps* Atkinson, *P. leoninus* Fries var. *coccineus* Massee.

This species is not only of surpasing beauty, but is also exceedingly rare, and on that account has been observed with great interest in a new station at Perry, Ohio. For several years it has appeared there on a lesion in the same red maple. The mycelium is without doubt well established in the diseased wood of this tree, but repeated search has failed to detect it on any other tree or in any other station.

It is certainly one of our most striking species. No agaric has more brilliant colors. Lange has recently collected and studied it in Denmark and considers it the same as Atkinson's *P. caloceps*. This conclusion seems to be reasonable, but study of our plant, and also of true *P. leoninus* Fries leads me to doubt the propriety of considering this brilliant plant a color form of *P. leoninus*. For reasons given below, it seems clearly distinct and to merit its own specific name.

Peck's keen eye early observed that the genus *Pluteus* can easily be separated into two sections by the nature of the surface of the pileus. One section has a silky appearance, and is more or less fibrillose, and the other has a "micaceous appearance" and is glabrous. Apparently he did not examine the cuticle of the two groups with a microscope, for he does not make clear the structural difference upon which the difference in appearance depends. Lange made this clear. In the first group the surface is made up of long interwoven hyphae whose pointed ends are sometimes more or less free so that the pileus is fibrillose, and sometimes wholly appressed so that the pileus is glabrous. In the other section the surface is made up of rounded cells, so that under moderate magnification it has the appearance of a cobble-stone

pavement. Under a microscope the difference is quite striking.

Pluteus leoninus Fries seems to be northern in its distribution. Kauffman reports it only from the "northern hemlock woods" of Michigan. Peck found it in the Adirondacks, and I have seen it only in Canada. It is lemon yellow but is larger than our common yellow species *P. admirabilis* Peck and has a solid stipe and silky pileus. When examined under the microscope the true species has the surface of the pileus made up of slender, interwoven hyphae exactly as Patouillard states. The scarlet plant, however, has the surface made up of rounded cells or in other words belongs to the micaceous section exactly as Lange has stated. The difference is plain to the naked eye, but under the microscope it is striking.

The scarlet *Pluteus*, then, differs from *P. leoninus* Fries not only in its color, but also in the structure of the epidermis of the pileus and is more closely related to *P. admirabilis* Peck and *P. sororiata* Karst. than to *P. leoninus* Fries. I believe it should be considered distinct under the name *P. coccineus* Massee. Apparently few botanists have seen both of these species living, which accounts for the confusion.

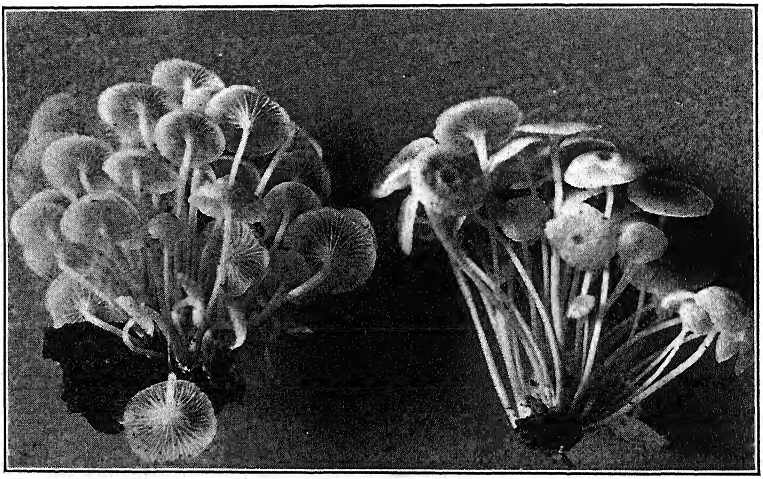
Mycena glutinosa sp. nov.

Pileo sub-membranaceo, campanulato-convexo, disco demum depresso, striatulato, glabro, viscoso, pellicula gelatinosa, secernibili tecto, albo; stipite albo, glutinoso, pellicula secernibili tecto, basi fibrilloso; Lamellis albis, angustis, non confertis, adnatis demum decurrentibus.

Pileus 1–3 cm. latus. Stipes 6–8 cm. longus. Sporae ellipsoideae $6–8 \times 3.5–4\,\mu$. Ad truncos, dense caespitosa Oviedo Fla. Cystidia, rara, parva, acuta.

This seems to be a very distinct species. It was found at Oviedo Fla., growing in dense masses on old logs. The plants are pure white and both pileus and stipe are very viscid, and both have a tough, gelatinous cuticle, which can easily be stripped off entire. The pileus is convex, and striatulate to the depressed center. The lamellae appear adnate at first but soon seem more or less arcuate decurrent. It could easily be considered an *Omphalia,* but in-so-much as several species of *Mycena* which are closely related to this plant are abnormal for *Mycena* in the same way it has seemed best to place this species with them.

Among a number of interesting species of *Russula* was one which was especially puzzling. Its appearance will be readily understood when it is said that it resembles *R. variata* Banning so closely in its narrow, crowded and forking gills, and in the blended colors of the pileus, that it was at once referred to that species when it was first observed. When it was closely examined, an interesting discovery was made. The spores were not only not like those of *R. varieta* but they were unlike the spores of any other species. They were thick-walled, smooth and nar-



Mycena glutinosa

rowly ellipsoidal so as to be almost cylindrical. *Russula ventricosipes* Peck certainly has smooth spores but the usual shape of spore in this genus is globose to round ellipsoidal. This spore difference was so marked that it seemed impossible that the plant could be a *Russula,* though an experienced collector from its appearance would at once refer it to that genus. During the past season this plant was found and studied again. The trama was found to have the typical vesiculose structure of *Russula* which places our plant in that genus in spite of its unusual spores. The diagnosis follows.

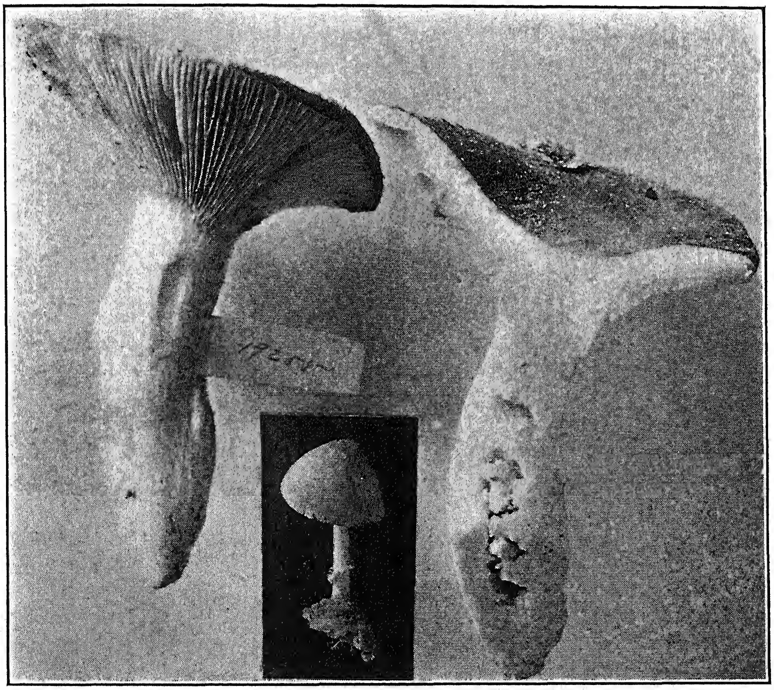
Russula heterospora sp. nov.

Pileo carnoso, rigido, convexo, demum depresso infundibuliformique, glabro, margine laevi et acuto, pallide carneo-roseo aut purpurino olivaceo commixto; stipite rigido, albo, glabro, solido, deorsum attenuato; lamellis attenuato-decurrentibus; angustis, confertis, furcatis; sapore et odore miti. Sporae laeves, albae, ellongato-ellipsoideae. 9–12 × 3.5–4.3 mc.

In nemoribus, Longwood Fla. *Russulae variatae* Banning simillima.

AMANITA PANTHERINA Fries

The occurrence of this species in the United States has seemed quite doubtful, although it has been reported several times. The



Russula heterospora
Inset Chamaeota pusilla Pat.

American species *Amanita cothurnata* Atkinson which is closely related to it is a common southern species, but it is consistently smaller and is pure white so that it is quite different in appearance.

What seem to be typical specimens of *A. pantherina,* however, have been found recently near Painesville, Ohio. They were ex-

actly like this species as Lloyd and I found and studied it in Sweden. The plants were robust, with the caps 10–12 cm. broad, and the stipes 10–14 cm. long and 2–2.5 thick, which is distinctly larger than is usual with *A. cothurnata* and instead of being pure white they were a deep sepia brown, with which the white fragments of the volva made a fine contrast. They had the stipe sheathed at the base and ellipsoidal spores 9–11 × 4–5 mc. which agrees well with the spores of both of these species, although the spores of *A. cothurnata* have been erroneously stated to be globose.

Whether *A. cothurnata* and *A. pantherina* are distinct specifically is a question on which mycologists will not agree, but at all events these Ohio plants are typical *A. pantherina* Fries and this species must be considered to belong in our flora, though it is doubtless rare.

PERRY, OHIO.

THREE NEW HETEROBASIDIOMYCETES

G. W. Martin

(with plate 31)

During the past few years I have examined a considerable number of tremellaceous fungi of which the characters do not seem to agree with those of any recognized species. Many of these have been collected by my students and myself; others have been sent in by correspondents. The confused state of the taxonomy of the group and the inadequacy of many of the descriptions make it desirable to use extreme caution in describing forms as new. The three species here presented seem, however, so clearly distinct as to justify description at this time.

Platygloea sphaerospora sp. nov. Plate 31, Figs. 1–2.

Late effusa, ceracea, margine indeterminato, separabilis, avellanea vel brunnea, hyphae tenues, 1.5–2 μ crass., dense intertextae; basidia cylindraceo-clavata, 25–30 × 6–8 μ, demum 60 × 5 μ, transverse 3-septata; sporae subglobosae, 7–8 × 5.5–6 μ.

Receptacle broadly effused, waxy, with indeterminate margin, separable, avellaneous to wood brown,[1] drying deep brownish red; hyphae slender, 1.5–2 μ in diameter, densely interwoven and more or less deliquescent; basidia at first swollen, clavate, 25–30 × 6–8 μ, later cylindrical-clavate, 60 × 5 μ, becoming transversely 3-septate, each cell producing a rather short epibasidium, 6–8 μ long; basidiospores subglobose, apiculate, 7–8 × 5.5–6.5 μ, germinating by repetition.

Type: G. W. M. *1222,* Dias Creek, Cape May County, N. J., Sept. 10, 1932, on rotten wood of *Quercus rubra* L. (*Q. falcata* Michx.). Another collection, G. W. M. 1191, same location and date and on same substratum.

Both fructifications were several centimeters in extent, but unfortunately only small portions were collected. No. *1222,* when moistened on October 20, 1932, shed spores freely.

[1]Indicates reference to Ridgway: Color Standards and Nomenclature.

Von Höhnel (Ann. Myc. **2**: 271. 1904) believed *Platygloea* Schröter 1887 to be a synonym of *Achroomyces* Bonorden 1851, and this view is adopted by Neuhoff (Bot. Archiv. **8**: 257. 1924). Reference to Bonorden's original description (Handb. Allg. Myc. 135) and to the slightly later description of *Achroomyces pubescens* by Riess (Bot. Zeit. **11**: 135. 1853) suggests that while this is not impossible, the fungi described by these early authors may well have been tuberculate fusariums. For the present, therefore, it seems preferable to use Schröter's generic name, particularly as the species here described is not erumpent and tuberculate, but broadly effused.

Sebacina sublilacina sp. nov. PLATE 31, FIGS. 3–10.

Effusa, ceracea, tenuis, hymenium pallidum, lilaceo-cinereum, sicca subinvisibilis, usque ad 7 cm. long., 1–2 cm. lat., in sectione 35–70 μ crass., cystidia fusiformia, 40–60 × 6–9 μ, probasidia subglobosa vel pyriformia, 7–10 × 6–7 μ, demum longitudinaliter septata; epibasidia 6–10 × 1.5 μ; sporae ovato-cylindracei, 6–7.5 × 3–4 μ.

Fructification waxy, broadly effused, separable when moist, very thin, indeterminate, forming irregular patches up to 7 cm. long and mostly 1–2 cm. broad, lilac gray to pale purplish gray [1] when moist, drying to an almost invisible film which appears as a faint lilaceous gray patch on the substratum; in section 35–70 μ thick, the densely packed basidia borne in tufts on longitudinal hyphae arising almost directly from the substratum, interspersed with tortuous, branched paraphysoids, which are apparently the branches which have borne basidia, and with scattered, thin-walled, hyaline, subfusiform, more or less tortuous cystidia, 40–60 × 6–9 μ, emergent for half their length and ending in a blunt, pointed tip; probasidia subglobose to obpyriform, 7–10 × 6–7 μ, becoming longitudinally septate into 4 or occasionally only 2 cells, mostly near the surface, hence the epibasidia rather short, 6–10 × 1.5 μ; spores ovate cylindrical, slightly curved, 6–7.5 × 3–4 μ, germinating by repetition.

Type: G. W. M. *1330,* Iowa City, Ia., Oct. 17, 1933, on dead oak branch. Not uncommon in Iowa, especially in late fall. Also Ohio, Missouri.

The prominent cystidia clearly mark this species as a member of the subgenus *Heterochaetella,* originally proposed by Bourdot as a subgenus of *Sebacina,* but later raised by its author to generic rank. Rogers (Univ. Iowa Studies Nat. Hist. **15**:[3] 9. 1933) points out

that the one character which distinguishes the group is not, in itself, of generic significance, and hence follows Rea and Burt in treating it as a subgenus. In this decision I concur. *S. sublilacina* differs from the other members of the subgenus in its very thin fructification, in the size and shape of the cystidia and in its spore dimensions.

Dacryomitra brunnea sp. nov. PLATE 31, FIGS. 11–14.

Tenaci-gelatinosa, gregaria vel congregata, 4–8 mm. alta; hymenophorum lobatum, contortum, 2–2.5 mm. latum, atrobrunneum; stipes crassus, sulcatus, basi pallidior; basidia clavata, 35–40 × 4–4.5 μ, epibasidia dua gerentia; sporae hyalinae, ovato-cylindraceae, uno latere depressae, demum 1-septatae, 9.5–12 × 4–5 μ; conidia ovata vel subglobosa, 3–4 μ.

Type: H. A. Kelly *773*, Parry Sound Region, Ontario, 1920, on coniferous wood. In herb. Univ. Mich.

Differing from all known species of *Dacryomitra* in the dark brown color as well as in the proportionately thick stalk and the flattened head. It is not, however, a *Dacryopsis*. Although flat, the head is definitely morchelloid and the stem is of the *Dacryomitra* type. When dry, the head is black and the stem dark reddish brown.

Since the tremellaceous fungi appear to have a not wholly deserved reputation as difficult to study, a word as to the methods used may not be out of place.

Whenever possible, a spore print should be secured. If the material is brought in from the field in expanded condition, provided it is neither immature nor over mature, all that is necessary is to wrap it in newspaper with the hymenium side down and in contact with a piece of black paper. For the larger fructifications a moist chamber is neither necessary nor desirable, but the smaller ones must be protected in some way against too rapid drying. If the specimen is dry when brought in, it must first be thoroughly soaked, preferably in distilled water, then dried on an absorbent surface, such as a towel, until the excess water is removed, and then wrapped in paper or placed in the moist chamber. Many species will give spore prints several weeks after being collected and dried in the laboratory. Such prints not only provide fully matured spores, but nearly always indicate the normal method of germination. Where this is by repetition, there may be several gen-

erations of secondary spores, those of each succeeding generation slightly smaller than those that gave rise to them, and due allowance for this fact must be made in interpreting spore measurements.

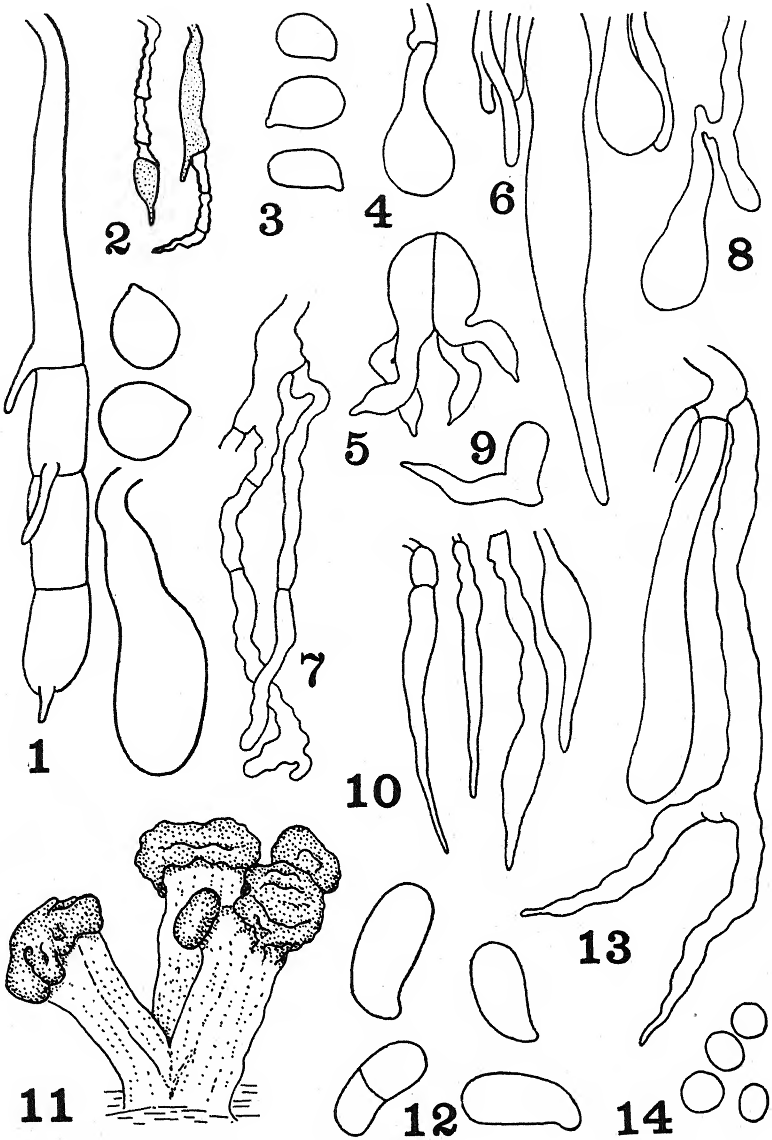
At least equally important is the fact that a fructification removed from a moist chamber or wrapping while shedding spores, and dried reasonably quickly in a moderately warm room will have many more basidia in various developmental stages than all but the most favorable collections as they come from the field. Excessive heat in drying should be avoided, as specimens which are dried too rapidly frequently fail to soak up properly when moistened later on.

The spore print is usually white, but may be pinkish or lilaceous, as in several of the commoner species of *Tulasnella,* or some shade of orange or yellow, as in many of the Dacrymycetaceae and a few species of *Tremella.* Where it is suspected that the spore color may not be white, part of the spore collection should be secured upon white paper, since the more delicate tints do not show up well against a black background.

As simple and elementary as these suggestions are, were they to be followed more generally in the collection and preservation of tremellaceous fungi, the material in our herbaria would be in far better shape for study than is now ordinarily the case.

For the demonstration of hymenial structures, staining is usually desirable. Our method has been to place either a thin section or a very small portion of the hymenium on a slide, drain off any water present, flood with a drop of alcohol, drain off the alcohol and quickly add a drop of 3 per cent aqueous potassium hydroxide and a small drop of 1 per cent aqueous Phloxine, mixing if necessary before putting on the cover slip. The hydroxide softens the material when it is tough or tenacious, permitting the separation of the hymenial elements by light pressure. Such a preparation usually fades in a day or so, but if the hydroxide is replaced by acidulated glycerine in proper dilution it makes a brilliant and permanent slide. For a denser stain, with indication of nuclei, Amann's medium with nigrosin is excellent.

For the determination of many important characters, sections are necessary. The waxy species present no difficulty. Con-



HETEROBASIDIOMYCETES

trary to general belief, however, the gelatinous species will provide very satisfactory sections if proper care is taken. A very sharp razor is needed and the material must not be too wet. If soaked and then dried until firm, good, thin sections may be secured in pith. Temporary distortion caused by pressure between the halves of the pith is ordinarily satisfactorily corrected by the hydroxide solution. Of course, for thin, entire sections of large fructifications, paraffin must be resorted to. In such cases dehydration by a butyl alcohol series seems to give better results than the use of ethyl alcohol.

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IOWA CITY

EXPLANATION OF PLATE 31

All microscopic drawings made with aid of camera lucida and reduced in reproduction to magnification indicated.

Fig. 1–2. *Platygloea sphaerospora.* Fig. 1, nearly mature basidium, probasidium and two spores, × 1500; Fig. 2, two basidia, the spores discharged from all but the terminal cell of that on the left, from all but the basal cell at the right, × 683.

Fig. 3–10. *Sebacina sublilacina.* Fig. 3, three spores to show variation in size and shape; Fig. 4, probasidium, with clamp connection at base; Fig. 5, mature basidium; Fig. 6, tip of cystidium protruding from hymenium, the latter showing a probasidium and paraphysoids; Fig. 7, tortuous, branched paraphysoids; Fig. 8, tortuous branch with swollen tip (probasidium?) ; Fig. 9, spore germinating by repetition. Fig. 3–9, × 1500. Fig. 10, four cystidia, to show variation, × 683.

Fig. 11–14. *Dacryomitra brunnea.* Fig. 11, habit, × 6; Fig. 12, four spores; Fig. 13, probasidium and collapsed basidium; Fig. 14, four conidia. Fig. 12–14, × 1500.

GODRONIA URCEOLUS AND OTHER CENANGIACEAE ON RIBES

EDITH K. CASH

(WITH PLATE 32 AND 1 TEXT FIGURE)

A collection of fungi made in Grand Mesa National Forest, Colorado, during the summer of 1930 by Mr. R. W. Davidson of the Division of Forest Pathology, was found to include three species of Cenangiaceae on *Ribes,* two of which were again found by Mr. Davidson in June 1933. One of these discomycetes has been identified as *Godronia urceolus* (Alb. & Schw.) Karst.; the others appearing to have been undescribed are here described as new species.

1. GODRONIA URCEOLUS (Alb. & Schw.) Karst. (PLATE 32, FIG. 1.)

Apothecia single or caespitose, substipitate, urceolate, membranaceous, .5–1 mm. diam. and height, dark greenish olive to olivaceous black,[1] exterior concentrically strigose from base to margin, wrinkled and paler at the margin; opening at first small, round, even, becoming lacerate, hymenium dark mouse grey; asci cylindrical, short pedicellate, slightly narrowed at the apex, 100–130 $\times$ 6–8 μ, usually about 110 $\times$ 7 μ; spores acicular, multiseptate, hyaline, acute at both ends, 66–75 $\times$ 1.5 μ; paraphyses filiform, branched near the tip; exciple composed of dark greenish olive, thick-walled, subcircular to elongate cells, with fascicles of darker hyphae in more or less regular streaks.

On twigs of *Ribes* (?*montigenum*), Grand Mesa National Forest, Colo., June 19, 1930, R. W. Davidson *363-a*; on *Ribes* sp., June 11, 1933, R. W. D. *772-a*.

The fungus agrees with the description of *G. urceolus* given by Rehm (7, p. 238) and with that of Albertini and Schweinitz (1, p. 332). In the illustration of the latter (PLATE 3, FIG. 4), the

[1] Color terminology follows that in Ridgway, Color Standards and Color Nomenclature, Washington, 1912.

apothecia appear sulcate, but it is clear from the description that the apparent furrows are in reality striae of dark hyphae. Nannfeldt (**5**, p. 283) suggests that *G. urceolus* is restricted to *Alnus,* and does not occur on other hosts. Rehm (l.c.) lists *Betula alba, Symphoricarpus racemosus,* and *Ribes rubrum* in addition to *Alnus,* commenting on the more caespitose habit on *Ribes.* A specimen of *G. urceolus* f. *Betulae* on birch (Rehm, Ascomycetes *1977*), examined by the writer through the kindness of Dr. F. J. Seaver, does not appear to differ specifically from the fungus on *Ribes.* A specimen on *Ribes prostratum* from Ontario, ex herb. R. F. Cain *1517,* determined by Mr. Cain as *G. urceolus* is also apparently the same species as the Colorado material, although the average spore length is only 55 μ.

An interesting problem was presented by a pycnidial fungus associated with the apothecia of the *Godronia* in the 1933 collection (no. *772-a*). This was determined as *Mastomyces uberiformis* (Fries) Karst. (*M. Friesii* Mont.), agreeing in the olive-brown to black pycnidia, emerging from a basal stroma and exuding masses of spindle-shaped, 3-septate spores, borne on simple or branched conidiophores, such as described by Petrak and Sydow (**6**, p. 368). The pycnidia in the Colorado fungus are depressed-globose rather than conical, and less prominent than in some well-developed specimens of *M. uberiformis,* but there seems to be considerable variation in shape at different stages of maturity.

M. uberiformis (Fries) Karst. has been stated by various authors, apparently on the basis of Fuckel's assertion, to be a stage in the life history of *Scleroderris ribesia* (Pers.) Karst. In order to test this assumption, herbarium specimens of the two fungi, chiefly exsiccati, were examined. In no case was the Mastomyces found in association with *S. ribesia. Fuckelia Ribis* Bon., recognized by Fuckel and others as a stage of this discomycete, was the only conidial form present with it in the following: Allescher and Schnabl Fungi Bavarici *170;* Fries Scler. Suec. *131;* Holl. Schm. and Kunze Deutschl. Schw. *73;* Jaczewski, Komarov and Tranzsch. Fungi Ross. Exs. *43;* Krypt. Exs. Mus. Palat. Vindob. *2029;* Migula Crypt. Germ. Aust. and Helv. Exs. *216;* Nannfeldt Fl. Suecica *1011;* Petrak Fungi Alb. & Bosn.

Exs. *161;* Sydow Myc. Germ. *495,* and Vestergren Mic. Rar. Sel *932.*

On the other hand, whenever an apothecial stage was found associated with *Mastomyces* on the *Ribes* specimens examined, it proved to be not *Scleroderris ribesia,* but *Godronia urceolus.* Several specimens of *Mastomyces* showed either conidia only, or conidia with immature apothecia, having neither asci nor spores, as was the case in Vestergren Mic. Rar. Sel. *1769* on *Ribes rubrum* from Sweden, also in a specimen on *Ribes* sp. from Ontario, coll. H. S. Jackson, Univ. Toronto *3048.* In three instances, however, the *Mastomyces* was found in association with mature apothecia of *Godronia urceolus,* the characters of both apothecial and pycnidial forms agreeing with those in the Colorado specimen on *Ribes:*

Brenckle, Fungi Dakotenses *217* (conidia) and *217-a* (apothecia), as *Scleroderris ribesia* (Pers.) Karst. on *Ribes floridum,* Nyland Grove, N. Dak., May 4, 1913.

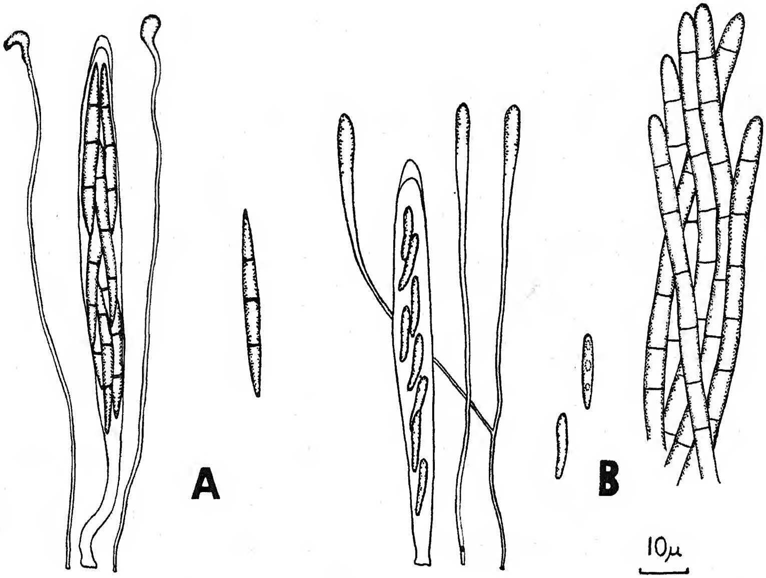
Phyt. Sect. Bot. Gard. U. S. S. R., *Mastomyces Friesii* Mont. socia *Godronia urceolus* Karst., on *Ribes nigrum,* Leningrad, coll. Vassiljevsky 5–16–1925.

Herb. Barbey-Boissier *2319, Mastomyces Friesii* Mont., on *Ribes nigrum,* Darmstadt, ex herb. Fuckel.

The apothecia in Fungi Dakotenses *217-a* have asci measuring $100 \times 6\text{–}7\ \mu$ and acicular spores $60\text{–}75 \times 1.5\text{–}2\ \mu$, and are clearly not *Scleroderris* as labeled. The third specimen is particularly significant, since it is on Fuckel's statement (3, p. 267) that is based the connection of *Mastomyces uberiformis* with *Scleroderris ribesia.* In addition to the Barbey-Boissier number *2319,* the label of this specimen bears the notation "F. Rhen. *1583,*" and is apparently a duplicate of the Fuckel specimen of that number, which is cited (l.c.) as the "macrostylosporiferus" stage of *Cenangium Ribis,* or *Scleroderris ribesia.* As shown in the accompanying illustration (PLATE 32, FIG. 2) *Mastomyces* and *Godronia* are present on the same stroma in this material.

Conflicting statements are found in literature as to the conidial stage of *Godronia urceolus.* Von Höhnel's *Chondropodium urceolus* on *Cornus,* with spindle-shaped, 1-septate spores, 52–68

$\times$ 3–4 μ (4, no. 958, p. 46) is obviously a different fungus. Brefeld, on the other hand, found associated with *Godronia* on *Ribes* conical, black pycnidia, containing indistinctly 3-septate spores, 26–30 $\times$ 3–4 μ, borne on branched conidiophores. Mycelium developing from these in culture was identical with that from ascospores of *G. urceolus,* with which fungus he concludes that it is connected (2, p. 290–291). Brefeld does not name his pycnidial fungus, but it is unquestionably *Mastomyces uberiformis.*



A, Godronia Davidsoni: ascus, paraphyses and spore: B, Scleroderris tumoricola: ascus, paraphyses, spores, and marginal hairs

Cultural studies will be necessary to establish definitely the connection between these pycnidial and ascosporic fungi on *Ribes,* but from the present evidence it seems probable that *Mastomyces uberiformis* will prove to be a stage in the life history of *Godronia urceolus* and not, as has been supposed, of *Scleroderris ribesia.*

2. **Godronia Davidsoni** sp. nov. (PLATE 1, FIG. 3; TEXT-FIG. 1, A.)

Apothecia sessile or substipitate, erumpent, single, depressed-globose to urceolate, .5–.7 mm. in diameter and height, membranaceous, buffy-olive to dark greenish olive, slightly strigose,

wrinkled when dry, with circular opening and fimbriate margin, hymenium smoke-gray; asci cylindrical, gradually narrowed toward the base and at the apex, 8-spored, 90–120 × 5–7 μ, usually about 110 × 6 μ; spores parallel or slightly twisted, acicular-fusoid, 3-septate, hyaline, 33–45 × 2.5–3 μ; paraphyses filiform, simple, hyaline, gradually enlarged to 2–2.5 μ and frequently recurved at the tip; exciple dark-pseudoparenchymatic at the base, with occasional dark olive brown striae, becoming paler toward the margin.

Apotheciis sessilibus vel substipitatis, depresso-globosis, membranaceis, olivaceo-brunneis, .5–.7 mm. in diam. et altitudine, hymenio ochraceo-griseo; ascis cylindraceis, base et apice angustatis, 90–120 × 5–7 μ; sporis acicularibus-fusoideis, 3-septatis, hyalinis, 33–45 × 2.5–3 μ; paraphysibus filiformibus, continuis, hyalinis, ad apicem gradatim incrassatis et curvatis, 2–2.5 μ diam.

On stems of *Ribes Wolfii*, near Mesa Lakes, Grand Mesa National Forest, Colorado, June 27, 1930, R. W. Davidson *460; Ribes bracteosum* X *R. nigrum,* Juneau, Alaska, July 12, 1923, J. P. Anderson *758*.

Superficially this species bears a close resemblance to *Godronia urceolus,* from which it may be distinguished by the flattened, smoother apothecia and the shorter, broader spores; the spores of *Scleroderris ribesia,* on the other hand, are broader and clavate. Judging from the description, *G. Andromedae* P. Henn. is a very similar fungus, but no material of this is available for comparison.

3. **Scleroderris tumoricola** sp. nov. (PLATE 32, FIG. 4; TEXT-FIG. 1, B.)

Apothecia sessile, usually caespitose, rarely single, erumpent on canker-like swellings, cupulate to nearly patellate when expanded, triangular or irregularly contorted when dry, coriaceous, wrinkled, furfuraceous, blackish brown to black with paler fimbriate margin, .5–2 mm. diam.; hymenium avellaneous or light drab, drying nearly black; asci cylindrical, narrowed at the apex, 8-spored, 90–100 × 5–8 μ; spores biseriate or more frequently irregularly uniseriate, unicellular, clavate, guttulate, acute at the lower end, 10–15 × 1.5–2 μ; paraphyses filiform, hyaline, simple or branched about half-way from the base, gradually enlarged to 2 μ at the apex; hypothecium hyaline to pale yellowish, 100 μ thick; exciple of dark-brown, parenchymatic cells, 5–7 μ in diam., roughened

with fascicles of septate, smooth, brown hyphae, paler toward the margin, $130 \times 3\text{–}5\ \mu$.

Apotheciis sessilibus, cupulatis vel applanatis, coriaceis, nigrobrunneis, furfuraceis, 1.5–2 mm. diam.; hymenio avellaneo; ascis cylindraceis, apice angustatis, $90\text{–}100 \times 5\text{–}8\ \mu$; sporis bi- vel uniseriatis, simplicibus, clavatis, $10\text{–}15 \times 1.5\text{–}2\ \mu$; paraphysibus filiformibus, hyalinis, apice $2\ \mu$.

On swollen, canker-like areas of twigs of *Ribes montigenum* found underneath the snow, Mesa Lakes, 9700 ft. elevation, Grand Mesa National Forest, Colo., June 13, 1930, R. W. Davidson *231* (type); on *Ribes* sp., June 11, 1933, R. W. D. *772-b*.

Spore septation in *Scleroderris* is extremely variable, dependent on the degree of maturity of the material. Long, clavate spores, like those in the fungus described, are frequently simple at first, with homogeneous contents, later becoming many-guttulate and remaining non-septate for a considerable time, but eventually showing one or more distinct septa. It seems preferable therefore, to place the fungus in *Scleroderris* rather than *Cenangium*, even though no septate spores have been observed. The presence of the fasciculate, septate hyphae suggests *Crumenula*, to which pilose species of the Cenangiaceae are usually referred; forms with more or less well-developed hairs are, however, included under other genera of the family and the limits of the genus are not clearly defined. Nannfeldt (**5**, p. 283) in the most recent treatment of the group, includes *Crumenula* as a synonym of *Scleroderris*.

Type specimens of the two new species have been deposited in the Mycological Collections of the Bureau of Plant Industry, the Herbarium of the New York Botanical Garden, and the Farlow Herbarium of Harvard University.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

EXPLANATION OF PLATE 32

Fig. 1. *Godronia urceolus* on *Ribes* sp., Davidson *772-a*, $\times 3$.

Fig. 2. *Mastomyces Friesii* and *Godronia urceolus* on *Ribes nigrum*, Herb. Barbey-Boissier *2319*, $\times 10$.

Fig. 3. *Godronia Davidsoni* on *Ribes Wolfii*, Davidson *460*, $\times 3$.

Fig. 4. *Scleroderris tumoricola* on *Ribes montigenum*, Davidson *231*, $\times 3$.

Photographs made by M. L. F. Foubert.

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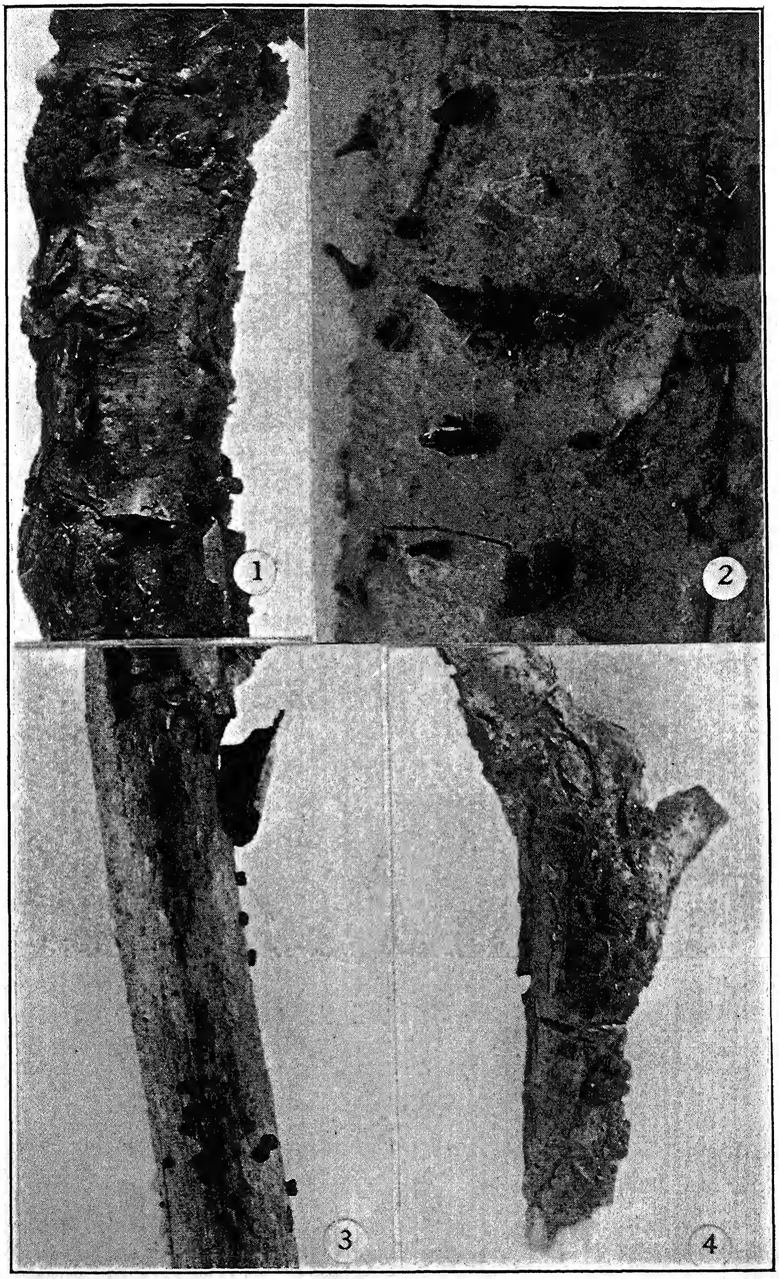


CENANGIACEAE ON RIBES

NOTES AND BRIEF ARTICLES

Wehmeyer's Diaporthe and its Segregates

In this volume, issued as the University of Michigan Studies, scientific series, volume 9, Wehmeyer treats the following genera, *Diaporthopsis, Apioporthe, Diaporthe, Diaporthella* and *Cryptodiaporthe.* The following new species are described: *Diaporthe dakotensis, D. Opuli, D. Bakeri, D. Fagi, D. Hickoriae, Cryptodiaporthe Macounii, Apioporthe Corni,* and *Diaporthopsis appendiculata.* The known species are described and many new combinations proposed. Of the three hundred and forty nine pages, one hundred are devoted to doubtful and unseen species. The volume contains much information on this important genus and it's segregates.

Buller's Researches on Fungi

Volume V of this well known work appeared January 3, 1934. The volume consists of two parts, the first devoted to hyphal fusions and protoplasmic streaming in the higher fungi, and the second to production and liberation of spores in certain non hymenomycetous basidiomycetes. The second part consists of three chapters. The first is a treatment of *Sporobolomyces,* a basidiomycetous yeast-plant and its spore discharge. The second chapter treats of the violent discharge of the basidiospores (secondary conidia) of *Tilletia Tritici,* and the third chapter discusses the *Sphaerobolus* gun and its range. The entire volume consists of 416 pages and is illustrated with 173 figures. The book is published by Longmans, Green and Co.

Another Rare Phalloid

Under the title "A Rare Phalloid From The New York Botanical Garden" (Mycologia 23: 83. 1931) the writer reported the occurrence of *Colus Schellenbergiae* Sumstine, in a very restricted area in the Garden where it had reappeared every season for a number of years. It was at that time suggested that it

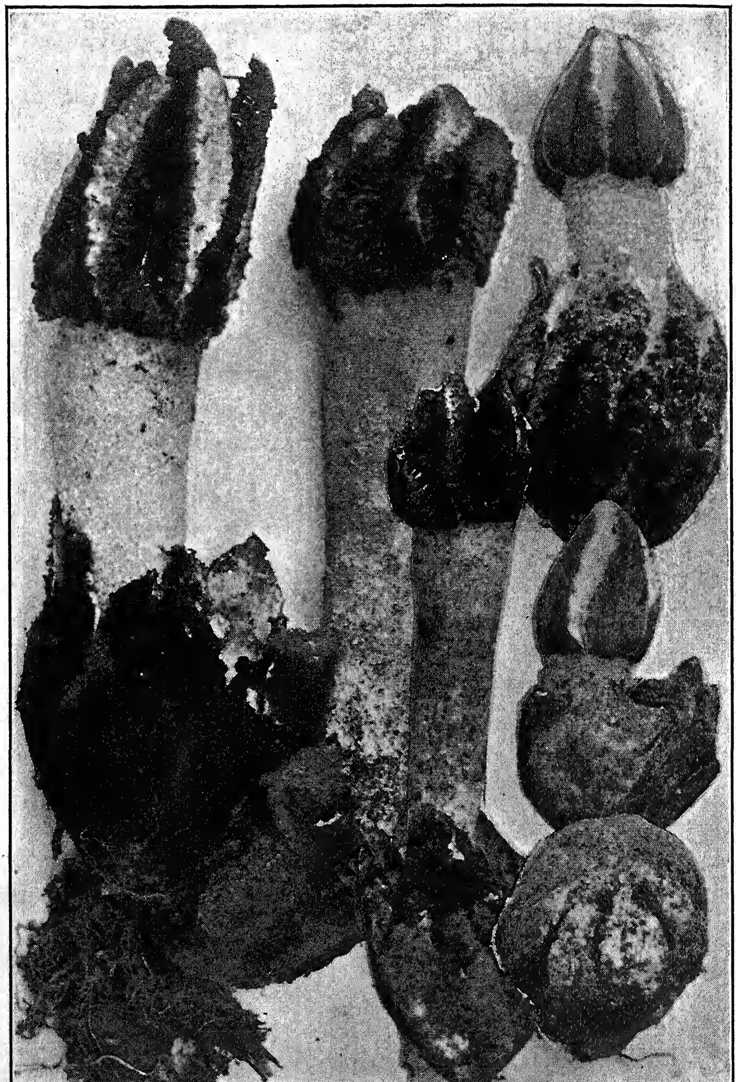


Fig. 1. Anthurus borealis

might be a synonym of *Colus javanicus* Penzig, reported from Java and this has since been found to be true. The only other known record for this species from continental North America is the collection from Pittsburgh from which Sumstine drew his description.

While it is a well known fact that many of the fungi are cosmopolitan in their distribution in the same latitude throughout the world, we ordinarily think of the tropical fungi as being more restricted. The appearance therefore of a supposedly tropical fungus in a northern latitude is of more than usual interest.

During the present season another rare phalloid was collected by Dr. A. B. Stout in one of his seed beds in The New York Botanical Garden. This was determined by the writer as *Anthurus borealis* and while more common than the preceding is still regarded as a rare fungus. A colored illustration of this species appeared in a early volume of Mycologia (**4**: PL. 68, FIG. 8). This was prepared by Dr. W. A. Murrill from material collected on Blackwell's Island in New York City. The species was described by Dr. E. A. Burt in 1894 from material collected in New York State. The name "*borealis*" apparently suggests that it is a northern species of what is usually looked upon as a tropical genus. It has since been several times collected in various parts of the East.

In 1920 (Mycologia **12**: 37) Dr. Murrill reported the species as having been collected by Mr. Kenneth Boynton in gladiolus beds in The New York Botanical Garden. The recent collection by Dr. Stout represents a second record of this species from The New York Botanical Garden. Since it apparently occurs at such rare intervals, or at least is rarely observed, it is worthy of record and illustration. The accompanying photograph (FIGURE 1) was made from material collected by Dr. Stout. The younger specimens were developed in moist chamber.—F. J. SEAVER.

A POISONOUS BOLETUS FROM OREGON

During the examination and identification of a species of *Boletus* the writer tasted and apparently swallowed a very small piece of the inner flesh. Three hours later typical symptoms of mushroom poisoning became apparent. Vomiting occurred frequently. Bil-

iousness was accompanied by severe stomach cramps and diarrhoea. A slight thirst was experienced but water could not be retained on the stomach. A physician was called and two hours after the first symptoms began, an injection of 1/50 grain of atropin was administered. At about the same time hot-towel packs applied to the affected region gave slight relief. Bismuth and tincture of opium given internally were soon expelled. The atropin was very rapid and effective in its action, taking less than five minutes to relieve the cramped condition, after which rest and quiet were possible. One vomiting spell occurred twelve hours later but was not severe. Coffee gave relief from thirst and was retained after this last attack. No bad effects other than weakness and sore chest muscles were apparent the following day. If a larger portion had been tasted, however, additional and more severe symptoms no doubt would have resulted.

These symptoms of mushroom poisoning were suggestive of those produced by *Amanita muscaria,* the fly agaric. A specimen sent to Dr. H. B. Myers of the University of Oregon Medical School for analysis, however, gave no evidence of a muscarin type of active principle by either chemical or biological methods.

Dr. S. M. Zeller kindly identified the *Boletus* under consideration which appears to be the western form of *B. satanus* of the *Luridi* group, known as *Boletus Eastwoodiae* (Murrill) Sacc. and Trotter. The original plants were very small due to a lack of soil moisture but later collections agree well with Dr. Zeller's notes on this species. The plants were found several times during September and October, 1933, in the Oak Grove district of the Hood River Valley, Oregon, being associated with trees of *Quercus garryana.*

This is believed to be the first definite report of poisoning by this fungus, although Murrill has previously classified it as suspected.

J. R. KIENHOLZ

U. S. FRUIT DISEASE FIELD LABORATORY,
HOOD RIVER, OREGON.

Mycological Society of America

THE SUMMER FORAY

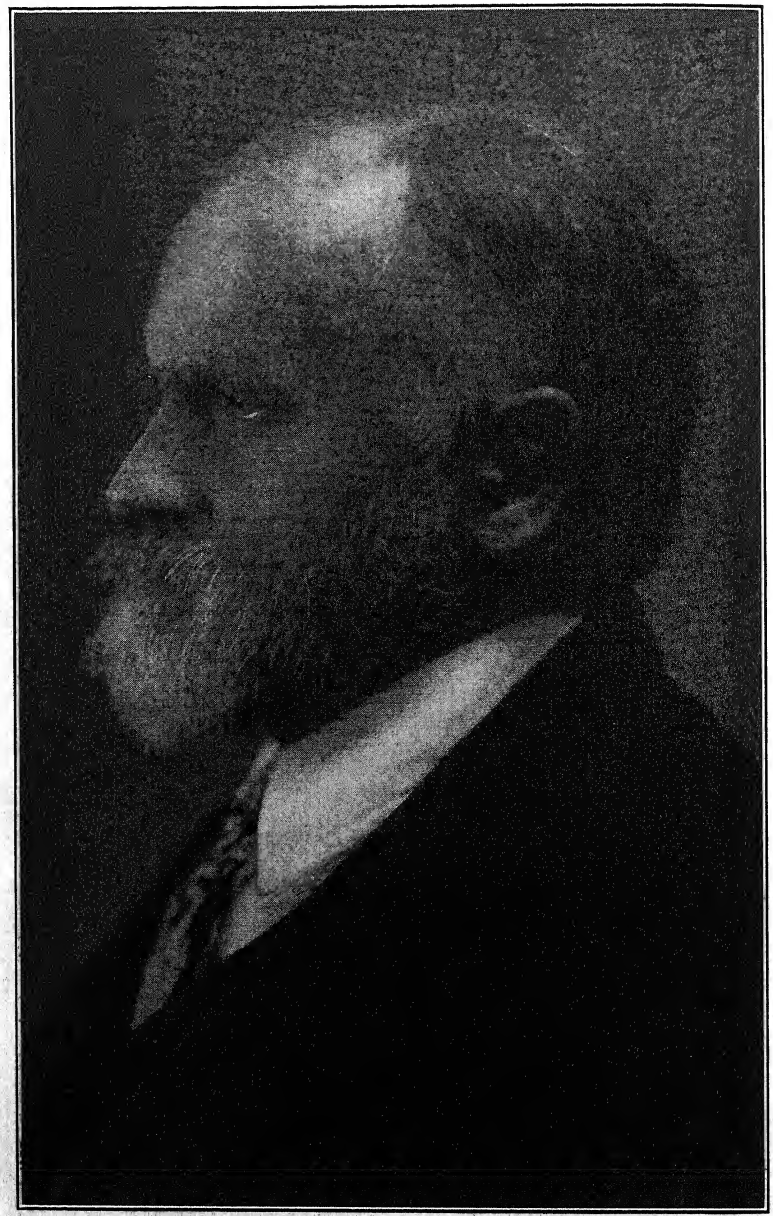
The second annual summer foray of the Mycological Society of America will be held at Seventh Lake, near Inlet, N. Y., in the southwestern Adirondack Mountains, August 21 to 24 inclusive. Headquarters will be at the camp of Professor F. C. Stewart. His boat-house will serve as our laboratory, and will be provided with a small working library, several microscopes, and a moderate supply of blotter driers and plant presses. Other items such as vasculums and baskets should be brought by those attending the foray.

Living accommodations can be obtained at hotels in the vicinity. The nearest railroad station is Thendara, 15 miles distant. Seventh Lake is one of the famed Fulton Chain, and has long been regarded as one of the most attractive of the many lakes in that region. It is surrounded by dense forest, and yet is available over splendid state roads. It lies considerably to the south of the highest of the Adirondacks, but these can be reached by automobile in a few hours. All who plan to attend the foray are asked to advise either the secretary-treasurer or Professor Stewart as far in advance as possible and make known what kind of accommodations they desire in order that adequate arrangements may be made. Both are thoroughly familiar with the region and will be glad to answer inquiries. Tenting is a possibility. Those thinking of using tents should obtain Recreation Circulars 2, 3 and 7 of the Conservation Department, Albany, N. Y. No charge is made for them. The Conservation Department furnishes also an excellent map of the Adirondack Mountains at a price of 15 cents.

Professor Stewart made the facilities of his camp available in the summer of 1931 at the occasion of a mycological foray held by American students of the Agaricaceae for Doctor Jakob E. Lange. In earlier years Professor Atkinson, Doctor Kauffman, and other prominent students collected there. A list of the higher fungi of the neighborhood has been kept which embraces about 670 species. The collecting when at its best is excellent, and in any August is good. Pleasant days and cool nights are guaranteed. There are no poisonous snakes, and at that season of the year few insects.

An easy climb to the summit of Black Bear Mountain nearby affords a pleasing panorama of the entire region. Members of the Society and other mycologists are urged to make careful note of the dates of the foray, and to arrange their summer plans to include it. This annual get-together in the field affords an opportunity for personal contact and exchange of ideas, and it may well develop into one of the most important of the Society activities.

B. O. DODGE, F. C. STEWART, H. M. FITZPATRICK,
Committee on Arrangements



Arthur Bliss Seymour

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XXVI JULY–AUGUST, 1934 NO. 4

ARTHUR BLISS SEYMOUR

1859–1933

L. R. JONES [1]

(WITH PORTRAIT)

The thinning ranks of the older school mycologists in America met another sad loss in the death of Arthur Bliss Seymour, March 29, 1933. This date lacks but a year of the full century following the death of de Schweinitz, American pioneer in this field. Seymour's work fully bridged the last half of this formative period in American mycology. Before, however, reviewing Seymour's

[1] The author is indebted to Professor Seymour's family for information obtained from correspondence, notes, etc., as to the ancestral and personal history. The bibliography is due especially to the daughter, Edith Seymour Jones.

In order to avoid, so far as might be, error concerning scientific facts and institutional relations involved, Professors William Trelease, J. J. Davis and W. H. Weston, each has kindly aided by a critical reading, with some minor revisions of the manuscript. Professor Trelease also invited attention to a contribution by Professor Seymour of definite and lasting significance which, as he suggests, well illustrates Seymour's peculiar genius for painstaking methods with meticulous attention to detail. At the Madison Botanical Congress of 1893 Seymour was member with Barnes and Britton of the committee on botanical bibliography and typography. Upon request Seymour prepared a report on the principles and rules which should govern citations to literature. This was adopted by the Congress and later by the Botany section of A. A. A. S. (see references in the bibliography). As a result "the Seymour rules of citation," in their essentials, have since continued to dominate the usage of most American botanical publications and have indirectly influenced bibliographical citations generally.

[MYCOLOGIA for May–June (**26**: 201–278) was issued June 1, 1934]

part in this half century's progress, it will be well to recall his unusual character and personality, and somewhat of that which contributed to these. The first American ancestor of his line, Richard Seymour, migrated from Hertfordshire, England, to Hartford, Connecticut, in 1639. Six generations of Seymours remained in Connecticut and Massachusetts until his own father went in 1853 from Granville, Massachusetts, to join other pioneers in Moline, Illinois. His maternal line came of similar New England stock. His mother, Mary Bliss, was, however, born in Hawaii, where her parents had gone as missionaries in 1837. Her father, upon returning, was pastor of the first church in Moline. Thus Seymour was of Puritan ancestry, "families of ministers and missionaries." Orphaned in early childhood, his boyhood upbringing by elderly great aunts and uncles fixed the inbred devotion to religious idealism and the direct values of the simple life. When that which is of best worth is clearly visioned one's course is fixed; it remains only to carry through as he did to the very end. Young Seymour was naturally a merry child, especially responsive to friendly companionships. It was the more unfortunate, therefore, that partial deafness resulted from scarlet fever before he was five years old. The consequent limitation in personal relations accentuated a natural tendency to shyness and introspection. These conditions doubtless contributed materially to his carefully considered devotion through life to a few major interests. His botanical studies were from their beginnings most inspiring and most exacting of time and energy. But they were to him an integral part of a life dedicated to what was conceived as his best worthwhile services to his kind. The spirit was the same whether expressed in devotion to family, to missions [2] or to mycology. Home and family responsibilities were at once his solace and concern throughout the half century of his professional life. Married in 1886, the parents left four children. In addition to the attendant home responsibilities Mrs. Seymour was a sympathetic co-worker in all his scientific undertaking until her death one year before his own. The first such service to mycology

[2] For more than twenty years he was teacher of a group of men in a Chinese Sunday School in Boston and in his later life a member of the Advisory Council of Yenching University, Peiping, China.

was in proof-reading the Farlow-Seymour Host Index, 1888–1889. The last, forty years later, was the far greater one of proof-reading the seven hundred exacting pages of the Seymour Host Index of 1929.

The instincts of the naturalist directed his boyhood bent to the plant life about him. Nothing could be more enticing, surrounded as he was by the lushest of varied floras where the Illinois prairies met the bottom lands of the Mississippi River. He early learned to identify the simpler plants from happy associations with a neighboring amateur botanist. His botanical interests in turn stimulated him to supplement his regular schooling by special work with Latin. And again this same interest in plants decided his choice of college. He might have entered Knox, near-by, but instead he turned in 1878 to Illinois University that he might be with Burrill. He took with him to Burrill's laboratory a herbarium of some 500 plants and his major interests continued in botany. The collegiate courses in this were limited so he soon completed these and became Burrill's assistant and tutor to the less apt.

From young Seymour's first contacts with Burrill the character and influence of this teacher continued a dominant influence in his life. Evidently this was true of many Illinois students during those two formative decades in economic botany, the seventies and eighties. Probably no other period in Burrill's half century at Illinois was more significant and stimulating than the six eventful years, 1877–1883, when Seymour was there. Burrill then initiated and completed his epochal demonstration that pear blight is caused by parasitic bacteria. He thus solved the riddle of a century in American horticulture and made one of the outstanding contributions to world progress in the field of plant pathology. Being a man of great energy and in life's prime, Burrill was at the same time pushing other important projects. His own teacher of botany, Sewall, being Curator of the State Museum of Natural History, Burrill had early become an enthusiastic leader in the botanical explorations of the Illinois Natural History Survey. Combining as he did responsibilities for leadership in horticulture with botany, and sensing the rapid increase in recognition of the importance of parasitic disease of plants as well as animals, he turned especial attention to the parasitic fungi of Illinois. As a result he

conceived of specialized surveys for these. From the outset, as Barrett [3] points out, Burrill happily associated his students with him in these natural history surveys extending "from Chicago to Cairo." Thus even as an undergraduate Seymour's early enthusiasm as a botanical explorer received constant stimulation from association with his veteran naturalist teacher.

Seymour's notes record that in the summer of 1879 he was commissioned to work over and systematize the valuable State Survey collections which had been accumulating in the University herbarium. The next summer while engaged in further field exploration, with increasing interest in the parasitic fungi, he first met F. S. Earle. This marks the beginning of their long and mutually stimulating associations in mycological work. Before graduation in 1881 Burrill assured Seymour of full time appointment on the staff of the Natural History Survey with opportunity to devote his time wholly to this fascinating work. He notes that "for nearly two years . . . I scoured the state with a hand lens as thoroughly as I could from Jo Daviess and McHenry to Union and Jackson Counties . . ." adding that when winter closed the field collecting, work turned to the compound microscope in the laboratory upon the fungi which he had collected, Burrill giving such aid as he could, and F. S. Earle coöperating. "Our leading topics at that time were the rusts and the powdery mildews," Seymour giving first attention to the rusts and Earle to the mildews. Thus the foundations were laid for the two monographs on the parasitic fungi of Illinois.[4] Doubtless had Seymour remained longer at Illinois he would have had the personal satisfaction and credit of joint authorship with Burrill on the Uredineae as had Earle on the Erysipheae. As it was, when Part I, dealing with the rusts, appeared in 1885 Burrill states in the Introduction, "most of the plants herein described were collected in Illinois in 1881 and 1882 by Mr. A. B. Seymour. The entire collection consisted of three

[3] Barrett, T. J., Thomas Jonathan Burrill. Phytopath. **8**: 2. 1918.

[4] Parasitic fungi of Illinois. Part I (Uredineae). T. J. Burrill. 1885. Part II (Erysipheae). T. J. Burrill and F. S. Earle. 1887. G. P. Clinton later continued in Burrill's laboratory this work upon the parasitic fungi, giving special attention to the smuts. His studies, completed in Farlow's laboratory, resulted in his monographs on American smuts. (See Seymour's note concerning this cited in the bibliography.)

thousand, seven hundred and eighty-four numbers, many of which are of course duplicated, or are different stages of the same species, leaving, however, a large number of distinct specific forms, much larger than is usually supposed to exist in our flora." Burrill, in the publication cited above, acknowledges also the efficient aid of Seymour in species determinations of the rusts aided by the facilities of the libraries and herbaria of the University and State Laboratory. In using these, Seymour records his early recognition of the need of indexes and the beginnings made with the card system in his student days. The usefulness of this card index was such that it grew rapidly during the winters concentrated upon mycological studies 1881–1883. This was to be of greater portent than at first conceived. As Seymour's notes record it, "in the spring of 1883 Professor W. G. Farlow, of whom I had been seeking some technical information, asked me to go to Cambridge as his assistant, temporarily. Burrill advised me to go. The need of a better library had been keenly felt by both of us, as had also facilities for using it. Cambridge had a smaller index of similar kind and greater facilities for extending it. It offered also greater opportunities for studying the plants themselves and that was what I wanted most. . . ." "Our purpose in Illinois was to know Illinois fungi. Dr. Farlow's purpose was to construct a monograph of North American Fungi."

The next year was spent busily and happily on the Harvard mycological indexes. This period, 1883–1884, marks the real beginning of his life work upon these indexes, but the permanent undertaking was destined to be delayed for three years by a series of distracting but eventful and profitable opportunities such as alert, venturesome young college graduates always welcome. The first of these was a return in 1884 for summer field work in mycology with Burrill and Earle, continuing the Illinois mycological surveys. The second immediately following in the autumn of '84 was a collecting trip over the Northern Pacific railroad to the Columbia River basin. Professor Forbes, as Director of the Illinois State Laboratory, had asked him to return the next summer for permanent appointment on the state staff. He would have so done but for the linkage of two other opportunities. In December '84 Dr. Farlow, wishing to join Dr. Gray on a winter's sojourn on

the Pacific coast, invited Seymour again to care for the herbarium and indexing during his absence. Immediately following, 1885–1886, he was called to the University of Wisconsin for a year. Professor William Trelease had, in 1881, gone there from Farlow's laboratory where he, just preceding Seymour, had also worked upon the Harvard Indexes. In 1885 Trelease transferred to St. Louis for the combined responsibilities at Washington University and the Missouri Botanical Garden. This led to the emergency opportunity for young Seymour to try his hand at teaching general botany at Wisconsin during the interim between the Professorships of Trelease and Barnes. He was urged to remain but although he recognized it as a stimulating and broadening experience, his natural genius, probably influenced by his handicap in hearing, confirmed his preference for specialized work in mycology rather than what he records as "the burdensome routine of teaching general botany." So he welcomed the opportunity which came in the autumn of '86 to accept Dr. Farlow's proffer of permanent appointment on the staff of the cryptogamic herbarium. This was the more opportune because in May of that year he had returned to Illinois for his marriage, and in June for his M.S. degree.

He was now adequately matured and trained for his special life work in mycology. The Harvard Indexes were to dominate the rest of his life. Before discussing these, however, some of the side interests, especially of the next decade, deserve reference. These serve to illustrate two life-long characteristics, his devotion in service to family and fellowmen and his naturalist's eagerness for the out-of-doors. The first specific side-service was suggested by the rapidly increasing attention to plant pathology consequent upon the organization of work in this field in the various state and federal institutions. Renewing his early Illinois associations with F. S. Earle, the two issued the eight fascicles of Economic Fungi (1890–1893) with later supplements; G. P. Clinton editing the Ustilagineae, which closed the series in 1905. The second side line came from his desire to have his children associated in nature study and consisted in supplying highly reliable botanical specimens and associated services to many colleges and schools. The children became eager and trained collectors and preparateurs and shared the joys of the naturalist with the satisfactions of small

material additions whether to the family educational funds or the budgets of foreign missions. Still a third undertaking, again combining his enthusiasm for the field with that for timely service, resulted in a summer school at Cambridge dealing with mycology, especially the parasitic fungi. The writer attended this in 1890 as one of an eager group of young college graduates. Professor Seymour proved an able lecturer, his talks characteristically enriched by blue-print outlines or synopses. The laboratory work dealt especially with systematic mycology. He was at his best in the field as an eager leader in assembling surprisingly rich collections of parasitic fungi, of which he knew both habitat and host relations over a wide area. At about this time also he was lecturing in botany in Radcliffe College. But it was no more his intent than that of Dr. Farlow to permit any such side lines to distract unduly from the dominant interest of both alike, the mycological indexes.

Seymour's work on the Harvard Mycological Indexes, begun as noted in 1883, continued from its resumption in 1886 without interruption until his last weeks of illness in 1933. Has there ever before been, may there ever again be, so great individual accomplishment in critical botanical indexing? Farlow's plan of 1874 could not possibly have anticipated the rapid rise in amount and diversity of literature to be reviewed or in uses to be made of such indexes. He must have conceived them as primarily for individual guidance in at once increasing personal understanding of the North American fungi and ultimately in preparing monographic publications. These purposes would have been suggested in his early associations with Gray and deBary, each of whom was in his own field the master botanical monographer of his time. Farlow's recognition of the need of such mycological monographing is shown in his preface to the Bibliographical Index where he notes the lack since those of deSchweinitz in 1822 and 1834. Doubtless the same idea had challenged other mycologists during the ensuing half century, but of these only one, Curtis, could have met it well. Curtis indeed so planned and his private herbarium was to have been the basis for this. It seems significant therefore that even while Farlow was with deBary in Europe in 1873, Dr. Gray purchased for him the Curtis herbarium. This nucleus of

the present Farlow herbarium awaited his return to Cambridge in 1874, with sets of the early European exsiccatae and the associated European literature, the beginning of the Farlow library. Here then at the very outset of modern American mycology Farlow began assembling the resources for such monographic studies, needing only the indexes to render these resources readily available. Seymour with Burrill's advice went, therefore, eagerly to join in the work with these, temporarily in 1883 and 1884 and permanently in 1886. Farlow was fortunately able to back his stimulating ideals by continuing throughout his life the enrichment of his herbarium and library upon which the indexing began and by final bequest providing a life annuity for Seymour as supplementing his Harvard salary. Farlow's support was matched, in turn, by Seymour's idealistic faith in the significance of this service to American mycology. To him the one regret was the forced relinquishment of personal researches upon the parasitic fungi. But whatever the task required was unstintingly rendered.

Thus the indexes grew apace with definite continuity in purpose and execution. Their wider usefulness became obvious with the emergence of economic mycology in the late eighties. Farlow at once responded to this with timely publications in the Harvard Library series. These opened in 1887, with the Farlow-Trelease "List of Works on North American Fungi," followed in 1888 by a supplement. Valuable as these were, the next in the series were even more so. In 1888 came Part I of the Farlow-Seymour "Provisional Host Index of the Fungi of the United States"; Part II followed in 1890 and Part III in 1891. Nothing of its kind could have been more timely or widely welcomed and therefore more encouraging to Seymour. Their reception seemed, indeed, but prophetic of far greater satisfactions to come if only he pushed his part in the work.

The card indexes were resolved into three interrelated parts, the author's index, the host index, and the species index. The publications had thus proceeded logically from the more exact and less bulky author's index to the host index. But the greater tasks with correspondingly greater significance lay in the yet unpublished species index. The widespread interest in and value of this became so obvious that the Carnegie Institution, in 1903, undertook

its publication. So great were the editorial burdens that actual publication was, however, delayed until 1905. Then appeared the first part of the first volume of Farlow's "Bibliographical Index of North American Fungi." This included all species alphabetically from *Abrothallus* to *Badhamia,* with exact citations to literature and carefully edited notes, chiefly by Farlow but some signed by Seymour. Farlow's preface to this not only indicated the continuance of this species index, but also stated that a new edition of the author's index was in preparation with additions up to 1905. For reasons unexplained, nothing more was published during Farlow's lifetime. Perhaps the experience with this single volume revealed unforeseen problems both as to labor and finance associated with the species index and these in turn inhibited the simpler tasks with the others.

The immediately pertinent thing in this article, however, is to record the significance of this situation to Seymour personally. Both the quantity and the complexity of the material to be indexed was increasing annually while his youthful vigor was waning. Therefore, the more trying was the necessity for persistance in the routine when the possibility of publication became ever more remote. Each new case of cards must add pro-rata to printing costs. In 1905 in the preface to the Bibliographic Index, Farlow estimated that up to 1903 about 150,000 references had been covered. When Part I of this appeared it seemed that on that date, 1905, its 305 pages might represent but little over five per cent of the total matter, which might then have filled over 5000 pages in 18 like volumes. What was each year to add to this? And yet the greater the growth, the more the need. In the preface Farlow states "The need of such an index has been felt in all our universities where the study of descriptive mycology is being pursued, as well as . . . experiment stations and other government establishments devoted to vegetable pathology. In the absence of such an index it has been necessary for different institutions to spend time and money in duplicating the work begun by us in 1874, and for a long time we have wished to publish our index . . . in order that it might be used by all. . . ." Farlow's statement of 1905 is far more pertinent today than then. The need as he defined it for the published index is universally felt. American

students of mycology, including all plant pathologists, are waiting and hoping that something may soon be done to carry out Farlow's wish of 1905, and make the species index generally available.

Fortunately the last decade of Seymour's life was made happier by the partial realization of his long-deferred hopes as to publication. In 1929 the Seymour Host Index of the Fungi of North America was issued by the Harvard University Press. As the writer in reviewing it then stated it represents " the seemingly impossible dream of many a youthful mycologist brought to realization." At least it meant that to Seymour; a partial reward for over 40 years of persistent work. Whereas the Farlow-Seymour Provisional Host Index of 1889–1891, with 213 pages, listed some 23,000 names of host and fungus, this final publication of 1929, with its 717 double-column pages, increases the citations of such names to some 80,000. The meticulous care in outlining his methods and rules in the Preface shows how consistently Seymour in this volume strove to maintain the exacting standards set by Farlow for the earlier series of Harvard Index publications. Seymour dedicated this last volume to the memory of William Gilson Farlow and records his indebtedness to Mrs. Farlow for constant interest in its preparation and material assistance in its publication. The dignity and worth of this Host Index is a constant reminder to American mycologists of the continuing need, as defined by Farlow thirty years ago, for the completion of publication of the author index and even more especially of the species index. When, and if, this need may be met and this long-deferred hope brought to realization, the dedication of the further volumes should be to Farlow and Seymour jointly that our enduring indebtedness may be thus acknowledged for the unique services to American mycology rendered by these two men.

In closing no fitter words are needed as to Seymour's part in this than those he inscribed on the fly leaf of the writer's copy of the Host Index; words which are an epitome of Seymour's life ideals—

> " A work of earnest friendship throughout.
> Keep the faith with those whom you can help."

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In addition to the above, Seymour wrote many short articles for popular magazines and a few notes in botanical periodicals, especially in the earlier part of his life. He also wrote chapters on spore plants in Bergen's "Foundations of Botany."

SOME NEW OR NOTEWORTHY FUNGI ON ERICACEOUS HOSTS IN THE PACIFIC NORTHWEST[1]

S. M. Zeller

(with plate 33 and 5 text figures)

Many plants belonging to the Ericaceae are native to the Pacific northwest and are subject to infection by a list of very interesting parasitic fungi as well as serving as host to many saprophytic species. A few of the new or noteworthy ones are discussed in this paper in which the Ericaceae is taken in the broader sense to include those plants which are placed by some botanists in a separate family, Vacciniaceae. The fungi are taken up alphabetically under the greater groups, in the following order: Ascomycetes, Basidiomycetes, and Fungi Imperfecti.

1. Cenangella Rhododendri (Cesati) Rehm.

On the seed capsules and stems of the inflorescence of *Rhododendron californicum,* on Still Creek, Clackamas county, Oregon, June. Collected by *C. R. Stillinger* and *L. N. Goodding.*

So far as the writer is aware, this is the first report of this species from America. The Oregon material answers Rehm's description of the European type very closely, except that the spores in ours are mostly 19–22 $\times$ 7 μ, while the descriptions give 15–20 $\times$ 4–6 μ. A few pycnidia of *Diplodina Eurhododendri* Voss were found associated, as reported in Europe. These pycnidia are 150–300 μ in diameter with pycnidiospores 11–14 $\times$ 2.5–3.5 μ.

2. **Dermatea brunneo-pruinosa** sp. nov.

Apothecia superficial, 0.6–2 mm. diam., closed at first then cup-shaped, sessile to short-stipitate; *exterior* powdery villose, fawn-colored to wood brown, because of brown, septate hyphae with swollen cells toward tip; *hymenium* fuscous black because of the

[1] Published as Technical Paper No. 212 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

brownish tips of the paraphyses which form an epithecium, 130–160 μ thick, hyaline below; *asci* hyaline, clavate-cylindrical, 120–140 × 10–14 μ; *spores* ellipsoid, hyaline, 1–2-guttulate, one-seriate, 14–20 × 7–10 μ; *paraphyses* slender, clavate, and light brown above, 4–6 μ broad at tip; *medullary* tissues a hyaline pseudoparenchyma, thick; *hypothecium* thin, of interwoven hyphae.

Type in O. S. C. Herb., *8096,* collected by F. P. McWhorter near Hauser, Coos county, Oregon, March 22, 1932.

On large dead spots on living leaves of *Gaultheria Shallon.* Common in western Oregon and Washington. This fungus is constantly associated with the leaf spot caused by *Pestalozzia gibbosa* Harkness and may always follow as a saprophyte on dead leaf areas produced by the latter. Since *Gaultheria Shallon* is affected by so many leaf spotting fungi throughout its distribution, however, and *Dermatea brunneo-pruinosa* is always found on the one caused by *Pestalozzia gibbosa* Harkness there is circumstantial evidence that the two are organically connected.

3. **Lachnum Gaultheriae** (Ellis & Ev.) Zeller, comb. nov. (syn. *Dasyscypha Gaultheriae* Ellis & Ev.).

On the under surface of a large leaf-spot of *Gaultheria Shallon.* Common, February to July (PLATE 33, FIG. 1).

The leaf-spot with which this fungus is associated is large and circular, 2–4 cm. in diameter, pinkish-gray to grayish-white above, rusty brown below, with a purplish-black, smooth border. In almost every particular this fungus is similar to *Lachnum echinulatum* Rehm. and possibly should be so referred. The latter, however, is a promiscuous, folicose saprophyte and *L. Gaultheriae* seems to be parasitic, constantly associated with a definite type of leaf-spot of *Gaultheria Shallon,* a host susceptible to several fungi, each of which causes a characteristic leaf-spot. Ellis and Everhart first described this fungus as *Peziza (Dasyscypha) Gaultheriae.*[2]

Another very closely related species of *Lachnum* should be mentioned in this connection since it is reported on an ericaceous host, *Vaccinium.* This was distributed by Ellis and Everhart[3] as *Pe-*

[2] Ellis, J. B., and B. M. Everhart. New West American Fungi. Erythea **1**: 199–200. 1893.

[3] Ellis and Everhart. North American Fungi No. *2144.*

ziza virginella Cooke and appears in Saccardo [4] under the name *Dasyscypha virginella* (Cooke) Sacc.

This is another typical *Lachnum* and is thus recombined as **Lachnum virginellum** (Cooke) Zeller, comb. nov. The spores are much longer than those of *L. echinulatum* Rehm. or *L. Gaultheriae,* but otherwise is similar to both.

4. LEPTOSPHAERIA GAULTHERIAE Dearn.

Common on ashy colored bark of dead stems of *Gaultheria Shallon*. The lesions are distinctly margined by a line.

5. **Lophodermium melaleucum** (Fries) De-Not. var. **epiphyllum** var. nov.

Maculae circular, ashy-gray, 3–4 mm. in diameter; *perithecia* epiphyllous, innate, oblong, about $600 \times 300\,\mu$, black, smooth, opening by longitudinal slits; *asci* long-cylindrical, $96–110 \times 7\,\mu$, 8-spored; *spores* filiform, hyaline to straw-colored, $60–67 \times 2–2.5\,\mu$.

On gray spots on living leaves of the evergreen huckleberry, *Vaccinium ovatum*. Waldport, Lincoln county. October.

This variety differs from the species in that the perithecia are always on the upper surface of the leaves. This is also true in *L. Oxycocci* but in that species the spores are much shorter.

6. MYCOSPHAERELLA ARBUTICOLA Peck.

On leaves of *Arbutus Menziesii,* Josephine county near Waldo, Oregon. February and June.

This is a very destructive parasite sometimes causing nearly a complete defoliation of the trees. (PLATE 33, FIG. 2.)

7. MYCOSPHAERELLA CHIMAPHILINA (Sacc.) House.

Causing leaf spot of *Chimaphila umbellata*. Common in western Oregon and Washington.

In our material the perithecia are $70–90\,\mu$ in diameter and the spores average $14 \times 4\,\mu$ and are hyaline. The western form then is not *M. Chimaphilae* Ellis & Ev. and is not synonymous with *Stigmatea Pyrolae* (Fries) Schröt. as given by Trotter.[5] The

[4] Saccardo, Syll. Fung. **8**: 444.

[5] Saccardo, Syll. Fung. **24**: 397. 1926.

perithecia are embedded in the leaf tissue, subepidermal; aparaphysate.

8. MYCOSPHAERELLA GAULTHERIAE (Cooke & Ellis) House.

On *Gaultheria Shallon,* Waldport, Oregon; April.

This fungus is associated with a very prevalent leaf spot, which detracts greatly from the beauty of this broad-leaved evergreen.

9. PHACIDIUM GAULTHERIAE Dearn.

On whitened spots on the stems of *Gaultheria Shallon.* Throughout the range of the host these characteristically white spots margined by a raised line may be easily identified.

10. PHACIDIUM VACCINII Fries.

On dead fallen leaves of *Rhododendron californicum,* Douglas county, Oregon; March.

The Oregon material conforms more nearly to the description of this species than any other. The asci are 45–63 $\times$ 7–8 μ and the spores are broadly lenticular, 10–11 $\times$ 3–4 μ.

11. RHYTISMA ARBUTI Phillips.

On leaves of *Arbutus Menziesii* throughout the range of the host. Earlier on the same leaves *Melasmia arbuticola* Vize. occurs, causing an unsightly appearance, especially to the everegreen leaves which have over-wintered. This is perhaps the most common leaf-spotting fungus on this host, doing considerable damage in southwestern Oregon.

12. CHRYSOMYXA PIPERIANA (Arthur) Sacc. & Trott.

Rust on leaves of *Rhododendron californica,* Clackamas and Curry counties, Oregon. April to September.

This rust was previously reported and illustrated by Schmitz and need not be discussed further here.

13. **Exobasidium Burtii** sp. nov.

Causing circular buff spots on the leaves of the small-flowered *Azalea* (*Rhododendron albiflorum*). Very common on this host throughout its range.

This is the species of *Exobasidium* which Burt[6] has discussed and placed with doubt under *E. Vaccinii.* He "referred here, with some doubt, the *Exobasidium* causing yellow-buff leaf spot galls on *Rhododendron albiflorum,* collected on mountains in Washington by W. N. Suksdorf. The basidia are 20–30 × 6 μ,

FIG. 1. Leaf-spot of *Rhododendron albiflorum* caused by *Exobasidium Burtii.*

with 4 prominent sterigmata; the basidiospores are mostly 18–21 × 4.5–6 μ, and are nearly all 3-septate. Some of these spores are germinating, hence the septation of the spores may possibly be due to their over maturity when collected, combined with

[6] Burt, E. A. The Thelephoraceae of North America IV. *Exobasidium.* Ann. Missouri Bot. Gard. 2: 627–658. 1915. (See p. 650.)

weather conditions at that time favorable to germination. Other collections which show the full series of gall forms on this host are desirable and should give the needed information in regard to septation of the spores."

Recently advantage was taken of an opportunity to study this disease on the Skyline Trail of the Cascade Range, from Olallie Lake to Breitenbush Lake, Jefferson county, Oregon. The observations were made the latter part of August, 1931, after a long, dry summer. Especial attention was given to the conditions mentioned by Burt but under all conditions the spores were found to be septate. Conditions were not at all conducive to spore germination, nor had they been for several weeks.

Since Dr. Burt hinted that this *Exobasidium* is perhaps distinct from *Exobasidium Vaccinii,* I take pleasure in dedicating it to him.

Leaf spot, yellow-buff above, lighter below, usually 1–2 cm. in diameter, flat; basidia hypophyllous, 4-spored, 18–28 × 5–7 μ; *sterigmata* stout, prominent; *basidiospores* early 3-septate (sometimes one cell septate lengthwise), 17.7–24 × 5–6.5 μ, hyaline, ellipsoid to allantoid; *conidia* abundant, hyaline, continuous, narrow cylindric, usually straight, 0.7–1.5 × 7–13 μ.

This species is between *E. Vaccinii* and *E. Vaccinii-uliginosi* in breadth of spores, and differs also from both in color and type of leaf spot and early septation of spores. A leaf spot of similar color but more brownish buff and with reddish margins, is caused by *E. Vaccinii* on *Vaccinium membranaceum, V. Myrtillus,* and *V. parvifolium.*

When the plants of *Rhododendron albiflorum* are well infected they appear variegated, as in text figure 1.

14. EXOBASIDIUM LEDI Karsten.

Forming purplish-gray, bladder-like galls on leaves of *Ledum glandulosum* Nutt., 12 miles northeast of Pierce, Clearwater National Forest, Idaho.

This collection was taken by J. R. Hansbrough and J. L. Mielke, July 31, 1931, and constitutes our only record of *Exobasidium* on this host in the northwest. It is morphologically similar to *E. Vaccinii* and doubtless Burt would include it there, but without cross-inoculation study I am using Karstens' name. The collectors say it is common and abundant in the Idaho locality.

15. EXOBASIDIUM PARVIFOLII Hotson.

Causing clavaria-like proliferations from canker-gall spots on stems of *Vaccinium parvifolium* Smith. Collected by the writer in Benton and Coos counties in June. Both collections were typical and very different from *E. Vaccinii* on the same host. This has been described in detail by Hotson.[7]

16. EXOBASIDIUM VACCINII (Fuckel) Wor.

This species attacks many different host plants belonging to the Ericaceae. In the Pacific northwest it occurs on the leaves of the Madrone (*Arbutus Menziesii*), the manzanitas (*Arctostaphylos manzanita, A. nevadensis, A. columbiana,* and *A. Uvi-ursi*), cultivated azaleas in greenhouse and gardens, *Cassiope mertensiana,* wild cranberry (*Oxycoccus oxycoccus intermedium*), Fool's huckleberry (*Menziesia ferruginea* and *M. glabella*), western *Rhododendron* (*Rhododendron californicum*), cultivated species of *Rhododendron,* and various species of *Vaccinium,* as *V. deliciosum, V. membranaceum, V. Myrtillus, V. ovalifolium, V. ovatum* (and its blue-berried variety, *V. ovatum saporosum*), and *V. parvifolium.*

In this listing of hosts I have followed Burt who has included all of these host genera in his list of hosts for this species providing the spore measurements conform with those of the typical *E. Vaccinii.* Burt perhaps on the same grounds would have included *E. Ledi* here, had he observed American material. Cross-inoculation may prove several species are included here as described in previous literature.

Suffice it to say here that collections on one or another host have been observed from all types of locations in the northwest from sea level to 3500 to 4000 feet elevations.

17. EXOBASIDIUM VACCINII-ULIGINOSI Bond.

This species occurs on several hosts in the Pacific northwest, although Burt[8] had but one American specimen to cite. This was what Burt calls a "shoot gall" and others have termed "rose bloom," on *Vaccinum membranaceum.* In this type of infection

[7] Hotson, J. W. A new species of *Exobasidium.* Phytopath. **17**: 207–216. *Illus.* 1927.

[8] Burt. Loc. cit.

characteristic of *E. Vaccinii-uliginosi,* all of the leaves above the point of infection are felty-white below and red or pinkish above. The mealy white felt below is made up mostly of basidia and basidiospores but on some hosts, especially *Rhododendron californicum,* many conidia are produced. The basidiospores are 7–9 μ in width, while in *E. Vaccinii* they are 2.5–4 μ wide and in *E. Burtii* they are mostly 4.5–6 μ wide.

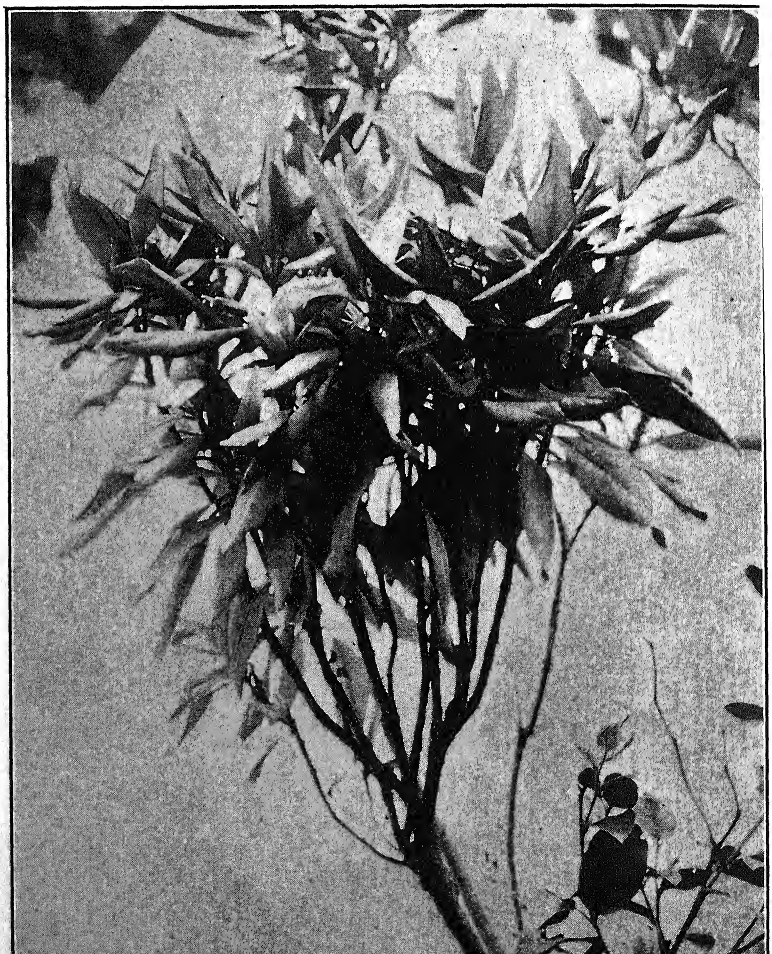


FIG. 2. Witches' broom of *Rhododendron californicum* caused by *Exobasidium Vaccinii-uliginosi.*

E. Vaccinii-uliginosi causes a distinct witches' broom with white (pinkish) leaves on *Rhododendron californicum.* Undoubtedly this is the witches' broom and white leaf to which Schmitz [9] has referred. This disease does considerable damage to the *Rhododendron* in the Cascade mountains. It is so characteristic in appearance that it cannot be mistaken (TEXT FIG. 2).

E. Vaccinii-uliginosi also causes a distinct witches' broom of *Vaccinium ovatum* (TEXT FIG. 3), and "shoot galls" of *Arctostaphylos columbiana* and *Phyllodoce empetriformis.* The disease of *A. columbiana* is very common and is typical of the European material such as distributed by Briosi & Cavara *261.*

FIG. 3. Witches' broom of *Vaccinium ovatum* caused by *Exobasidium Vaccinii-uliginosi.*

18. FOMES ARCTOSTAPHYLI Long.

Causing serious heart rot of *Arctostaphylos columbiana* and *A. patula,* east and west of the Cascade mountains in Oregon.

Since Long included no illustrations of this fungus with the original description [10] I am taking the liberty to include here one showing a sporophore on *A. patula* (TEXT FIG. 4) and the type of decay produced (TEXT FIG. 5).

[9] Schmitz, H. Observations on some common and important diseases of the *Rhododendron.* Phytopath. **10**: 273–278. *Illus.* 1920. (See *Pl. XI, fig. 3.*)

[10] Long, W. H. Three undescribed species of polypores. New Mex. Chap. Phi Kappa Phi, Papers **1**: 1–3. 1917.

Although Murrill has reduced this form to synonymy with *Fomes igniarius,* I am inclined to Long's viewpoint—that it is a distinct species. It is smaller than *F. igniarius,* produces a distinct rot which does not have the delimiting, definite black line, and grows differently in culture. Setae have not been found in the Arizona nor Oregon specimens while *F. igniarius* has setae at least sparsely distributed in the hymenium. This is a very common rot of manzanita in southwestern and central Oregon where it is difficult to find plants which are not affected.

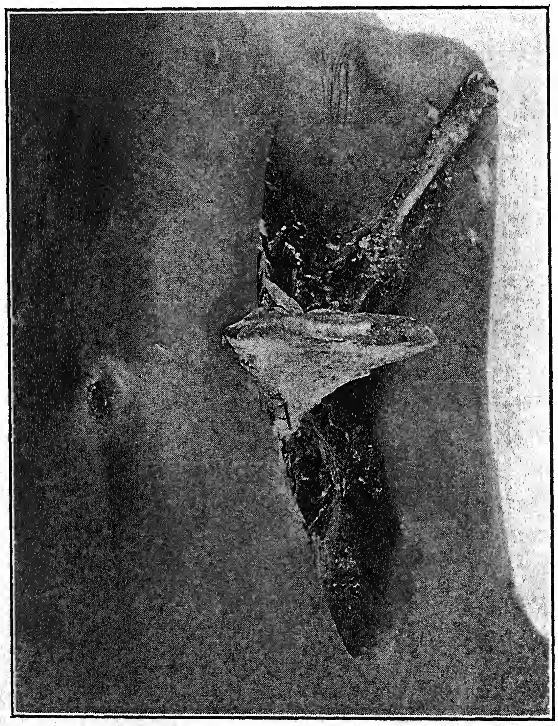


FIG. 4. *Fomes Arctostaphyli* Long on *Arctostaphylos patula.*

19. CRYPTOSTICTIS ARBUTI (Bonar) Zeller.[11]

Syn. *Disaeta Arbuti* Bonar, Mycologia **20**: 299–300. 1928.

Associated with a leaf spot of *Arbutus Menziesii* from Josephine to Douglas county, with a leaf spot of *Ledum glandulosum* Nutt.

[11] Zeller, S. M., and J. W. Deremiah. An anthracnose of *Ledum* caused by a species of *Elsinoë.* Phytopath. **21**: 972. 1931.

all along the west coast of Oregon, and with a leaf spot of *Arctostaphylos columbiana* from Canyonville, Douglas county, and Waldport, Lincoln county, Oregon. May to December.

This species was described by Bonar from material collected in Alameda and Marin counties, California. The new genus, *Di-*

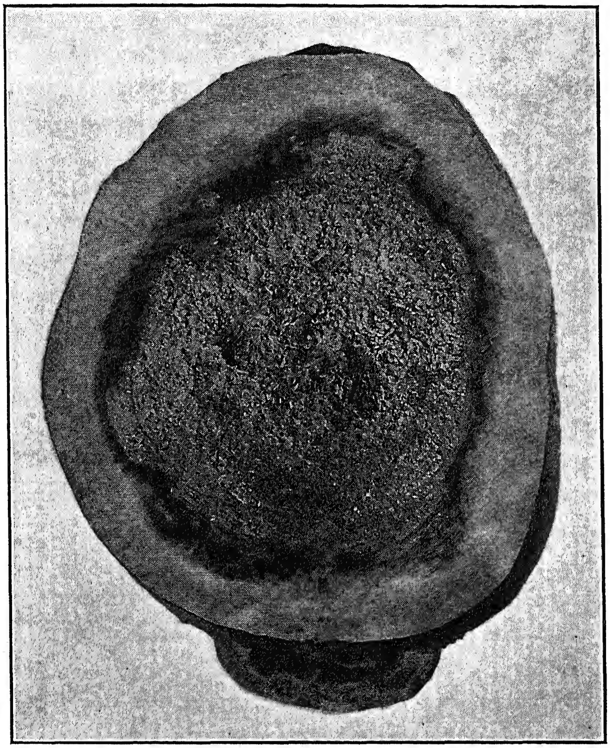


FIG. 5. Rot of *Arctostaphylos* wood caused by *Fomes Arctostaphyli.*

saeta, was based on this species, but a study of type material collected on Mt. Tamalpais, California, shows this to be typical of *Cryptostictis.* The material on *Arbutus* collected in Oregon agrees in every way with the type material and it is believed that the collections on *Ledum glandulosum* and *Arctostaphylos columbiana* are sufficiently similar to be referred here (PLATE 33, FIG. 3).

The leaf spots on *Ledum* and *Arctostaphylos* are mostly circular or occupy a whole leaf tip, 5–12 cm. broad, zonate with central

zones, grayish to light brown, and marginal zones dark brown, fringed with purplish tones. The fungus itself is as found on *Arbutus*. It may be that the setae of the spores are more flexuous in specimens from *Arbutus,* where they are often as long as the spores. On the three hosts the spores are constantly 4-septate.

Another species of *Cryptostictis* on an Ericaceous host in Oregon is *C. Mariae* on *Rhododendron,* reported below. This species differs from *C. Arbuti* chiefly in the 5-septate spores. Otherwise there could be little cause for designating them as separate species.

20. CRYPTOSTICTIS MARIAE (Clinton) Sacc.

Associated with a leaf spot of *Rhododendron californica,* throughout the range of the host in the Pacific northwest. January to October.

This fungus is constantly associated with a characteristic leaf spot of this host in the northwest. The spots are brown, being lighter toward the center, giving a "bull's eye" effect. This is exaggerated by the concentric lines of acervuli of the fungus. The spots are 4–25 mm. in diameter and when plentiful are ruinous to the appearance of this beautiful, wild shrub (PLATE 33, FIG. 4).

The Oregon material is identical in morphology to cotype material (Forestburg, N. Y.) kindly loaned by Dr. H. D. House.

Undoubtedly *C. Mariae* is the species which Schmitz [12] has mentioned as the cause of a leaf spot of *Rhododendron* and it is the disease which has usually been referred to as the *Coryneum* leaf-spot caused by *Coryneum Rhododendri* Cooke. This mistake is easily made when dried material is examined. Under such conditions during the preparation of microscopic mounts the brittle setae are broken from the spores. On the other hand, if fresh material is examined or if dry material is previously moistened with water for a few minutes before disturbing, the setae of the spores will usually remain intact.

Coryneum Rhododendri Cooke doubtless occurs in the northwest, as mentioned by Schmitz [13] but we have not seen it. The material referred to by the writer [14] in 1929 proves to be *Cryptostictis Mariae*.

[12] Schmitz, H. Some common and important diseases of *Rhododendron.* Phytopath. **10**: 277–278. 1920.

[13] Loc. cit.

[14] Zeller, S. M. Oregon fungi. Mycologia **21**: 109. 1929.

21. **Didymosporium arbuticola** sp. nov.

Maculi brown in center, purplish to reddish margins, 3–6 mm. in diam., sometimes coalescing; *acervuli* maculicolous, hypophyllous, circular, 130–240 μ broad, erumpent, breaking with a stellate margin of grayish epiderm; *conidiophores* hyaline, short, simple; *conidia* acrogenous, oblong-ellipsoid, usually straight, 1-septate, slightly constricted at septum, sometimes 3-septate, dark brown *en masse,* light brown or olivaceous under lens, $19–29 \times 7–8\ \mu$ (ave. 23×7.25).

On living leaves of *Arbutus Menziesii,* Corvallis, Oregon. December. (Type in Oregon Agr. College Herb. *4891.*) (PLATE 33, FIG. 5.)

It may be that since some spores become 3-septate this species should be referred to *Coryneum* but I have referred it to *Didymosporium* because most of the spores seem to be 1-septate and the 3-septate spores are usually constricted at the middle septum but not at the other two. Perhaps folicose forms like this should be referred to Spegazzini's genus *Phaeomarsonia.* Three-septate spores up to 35 μ long have been occasionally observed (PLATE 33, FIG. 6).

22. MELASMIA MENZIESIAE Dearn. & Bartholomew.

On leaves of *Menziesia ferruginea,* Tillamook and Clackamas counties, Oregon. Late summer.

A very interesting "tar spot" fungus.

23. PESTALOZZIA GIBBOSA Harkness.

On large zonate spots of living leaves of *Gaultheria Shallon.* Common in the coastal zone of western Oregon and Washington.

The leaf spot caused by this fungus is very large, often involving most of the leaf surface. They are typically zonate, the central portion grayish encircled by a light brown band which is bordered by a dark purplish broad line.

The spores of the fungus are very characteristic being 4-celled with one large dark cell and the appendages with knobbed extremities.

On the spots caused by this fungus *Dermatea brunneo-primosa* Zeller occur early the following spring.

24. PHYLLOSTICTA RHODORAE (Cooke) Tass.

Causing large brown spots on the leaves of *Rhododendron californica,* Mud Creek, and Shell Rock Lake, Clackamas Co., Oregon, June and July. Collected by L. N. Goodding and A. L. Hinkley, and G. D. Darker and L. N. Goodding, respectively.

In this collection the pycnidia are 100–160 μ in diameter and the spores are ellipsoid and $3.5–4 \times 2–2.5\,\mu$. In these respects it also answers the description of *Coniothyrium Rhododendri* P. Henn. but in this Oregon material the spores are colorless. The spots with which this fungus is associated are large and brown like a sun scorch and have the irregularly wavy curved margins so often seen when leaves are scorched. The pycnidia are black dots on these brown areas.

25. SEPTORIA SOLITARIA Ellis & Ev.

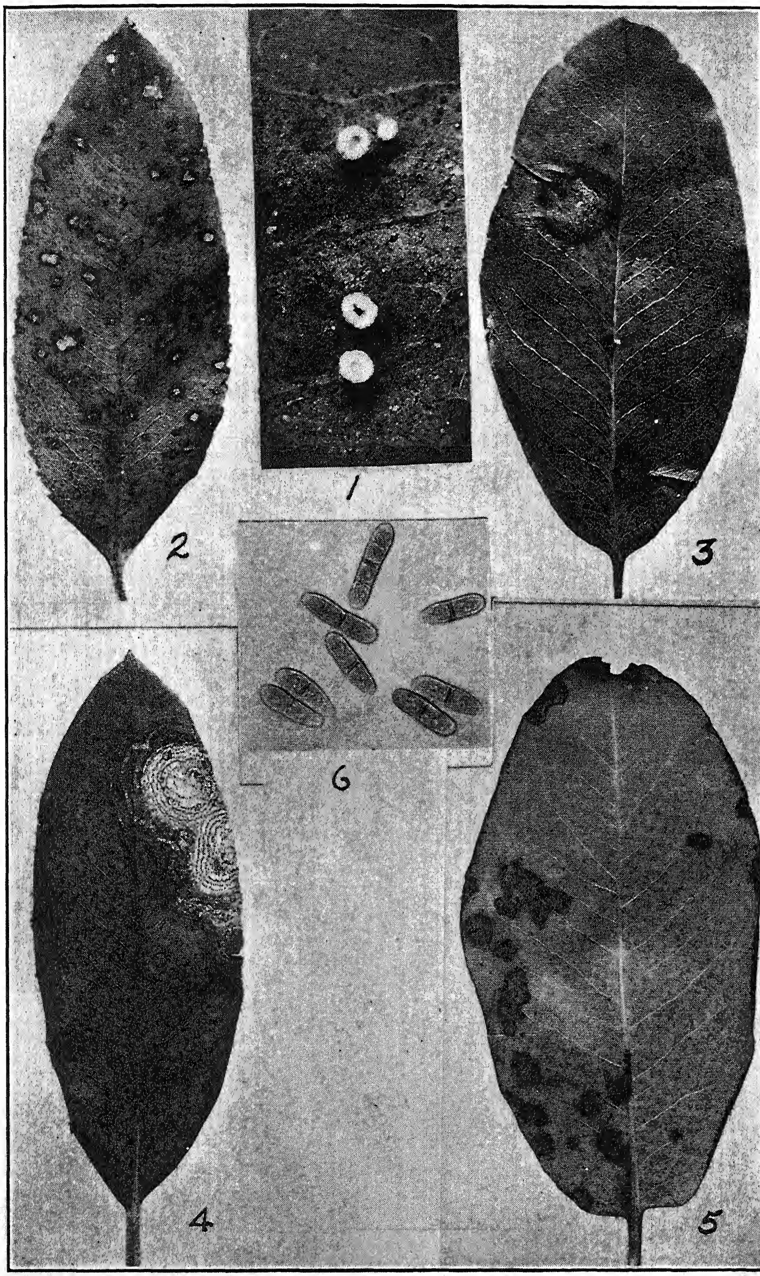
On leaves of the western azalea, *Azalea occidentalis,* along the coast, Coos and Curry counties, Oregon. July. Coll. *L. N. Goodding.*

This is perhaps the same species of *Septoria* which Ellis and Everhart so inadequately described. The spores in the Oregon material vary from 18 to 53 μ in length but they are mostly 35–50 μ, while those of *S. solitaria* were described as $20 \times 2\,\mu$. The name, however, is very appropriate for our material in which most of the leaf spots have a solitary black pycnidium standing out rather conspicuously on the ashy white background. The leaf spot is rather serious in certain locations.

OREGON STATE COLLEGE,
CORVALLIS, OREGON.

EXPLANATION OF PLATE 33

Fig. 1, *Lachnum Gaultheriae* on leaf of *Gaultheria Shallon,* $\times$ 8; 2, leaf-spot of *Arbutus Menziesii* caused by *Mycosphaerella arbuticola;* 3, leaf-spot of *Arbutus Menziesii* caused by *Cryptostictis Arbuti;* 4, leaf-spot of *Rhododendron californicum* caused by *Cryptostictis Mariae;* 5, leaf-spot of *Arbutus Menziesii* caused by *Didymosporium arbuticola,* showing lower leaf surface; 6, spores of *D. arbuticola,* $\times$ 400.



FUNGI ON ERICACEOUS HOSTS

INVESTIGATIONS OF TWO-SPORED FORMS IN THE GENUS MYCENA[1]

Alexander H. Smith

(with plates 34–38)

INTRODUCTION

It has long been known that within the genus *Mycena* there exists an unusually large number of forms bearing basidia which produce two instead of the usual four spores. In recent years even more variation has been noticed (Oort, **19**). In general the use of the two-spored basidium as a character of taxonomic importance in the Agaricaceae has been questioned. There still seems to be a difference of opinion, however. Lange (**13, 14**) by using this character in keying out species of *Mycena* and *Omphalia,* and in describing a two-spored species of *Tricholoma* has given it considerable weight. Kauffman in his unpublished manuscript on *Mycena* had also placed considerable emphasis on the two-spored character, chiefly it seems, because in *Mycena* an increase in spore size usually accompanies the change from the four- to the two-spored basidium.

In comparatively recent times considerable emphasis has been given to cytological and genetical studies of two-spored forms in the Agaricaceae and related families. The investigations of Bauch (**1, 2**), Buhr (**4**), Fries (**5, 6**), Harper (**7**), Juel (**8**), Kühner (**10, 11, 12**), Lewis (**16**), and Sass (**20, 21**) have given much information concerning the types of nuclear behavior in scattered species throughout most of the families of higher Basidiomycetes. In most species with two-spored basidia it has been found that there is a fusion of two primary nuclei in the basidium and that in the Agaricaceae, as a rule, two divisions follow before the spores mature. In the Clavariaceae three divisions are usually found. In the Agaricaceae, depending on the species, all of the four nuclei

[1] Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 455.

may migrate or two may degenerate in the basidium. A few forms have been described which are parthenogenetic. In the latter the young basidium contains only a single nucleus.

Since no intensive study had been made involving both those species which are known to have four- and two-spored forms, and those isolated species in which only two-spored basidia are known, it seemed very desirable to investigate as many of both types as could be found in a single genus in order to furnish, if possible, a better understanding of the position of two-spored forms in the classification of agarics, and in addition to obtain information concerning the nuclear history in a large number of closely related variant forms. The genus *Mycena* seemed to be a particularly favorable group of species for such an investigation because of the relative abundance of material, and because of the fact that both of the types of nuclear history mentioned above had previously been found among its species.

The writer wishes to express his appreciation to Professor E. B. Mains for advice and guidance during the course of the present investigation, and to Professor W. R. Taylor for his interest and helpful suggestions in the cytological studies.

MATERIALS AND METHODS

Since few cytological investigations have previously been made on species of *Mycena, M. alcalina* Fries, *M. Atkinsoni* House, *M. hemisphaerica* Peck, *M. haematopoda* Fries, *M. leptocephala* Fries, *M. murina* Murr., *M. sanguinolenta* Alb. & Schw., *M. stannea* Fries and *M. viscosa* Maire were studied in order to establish the usual type of nuclear behavior. The following nineteen variant forms were investigated: *Mycena alcalina* Fries, *M. capillaris* Fries, *M. citrinomarginata* Gillet, *M. clavicularis* Fries, **M. cholea** sp. nov.,[2] *M. graveolens* Kauff. & Smith, *M. immaculata* Peck, *M.*

[2] **Mycena cholea** sp. nov.—Statura Mycenae vitilis; pileus 2–12 mm. latus, obtuse conicus demum campanulate expansus, fuscus vel bruneolus, expallescens, striatus, non pruinosus; lamellae subconfertae, adnatae, albidae interdum pallide sordidae; caro incisa demum incarnata, sapore quininae simili, praecipue in stipite succosus, succo lacteo non copioso; stipes (1) 3–6 (10) cm. longus, 1 mm. crassus, fuscus demum pallidus; sporae 9–11 (12) $\times$ 6–7.5 (8) μ, ovoideae; basidia bispora; cystidia numerosa, apice cuspidata, 55–80 $\times$ 10–14 μ. Specimen typicum in Herb. Mich. conservatum, A. H. Smith n. 32–545, prope Ann Arbor, Mich. Oct. 8, 1932.

lasiosperma Bres., *M. dissiliens* Fries, *M. leptocephala* Fries, *M. margaritispora* Lange, *M. megaspora* Kauff., *M. metata* Fries, *M. mirata* Peck, *M. polygramma* Fries var. *albida* Kauff., *M. roseipallens* Murr., *M. rubromarginata* var. **Laricis** var. nov.,[3] *M. viscosa* Maire, and *M. vitilis* Fries.

The cytological studies have all been made from fruit-bodies collected in the vicinity of Ann Arbor. Because many of the species in this genus are very small and delicate it is desirable to kill and fix them for cytological study in the field when they are collected. In killing such material it is necessary to remove the air from between the lamellae in order to obtain the best results. It was found that by reversing the leather on the plunger in an ordinary tire-pump, and fitting the rubber tube with the proper connections, a very effective apparatus could be made. It gave rapid evacuation without damage to the delicate lamellae, and could conveniently be carried into the field.

The most satisfactory killing solutions were those of Flemming in various dilutions, and the following two known as Allen's modification of Bouin's solution.

1. After La Cour (15)	
Picric acid, sat. aq. sol.	75.0 cc.
Formalin	25.0 cc.
Acetic acid (Glacial)	5.0 cc.
Urea	2.0 gms.
Chromic acid	1.5 gms.
2. Picric acid, sat. aq. sol.	75.0 cc.
Formalin	25.0 cc.
Acetic acid (Glacial)	5.0 cc.
Urea	1.0 gm.
Chromic acid	0.5 gm.

The washing, dehydrating, and embedding were carried out in the usual manner. Sections were cut at thicknesses varying from

[3] *Mycena rubromarginata* var. **Laricis** var. nov.—Pileus 5–20 mm. latus, obtuse conicus, demum expansus et interdum late campanulatus vel convexus, glaber, striatus vel plicato-striatus, vinaceo-umbrinus, expallescens; lamellae pallidae vel cinerae, subdistantes vel distantes, adnatae, acie rubeola vel concolore; stipes pileo concolor, 2–4 cm. longus, 1–1.5 mm. crassus, glaber, fragilus; sporae 10–12 $\times$ 5–7 μ, subpyriformae; basidia 26–28 $\times$ 6–8 μ, bispora; cellulae aciei lamellarum subfusoideo-ventricosae, 40–75 $\times$ 5–12 μ. Hab. ad truncos laricinos. Specimen typicum in Herb. Mich. conservatum, A. H. Smith n. 32–229, prope Ann Arbor, Mich. June 17, 1932.

two to fifteen microns, depending on the tissue which was to be studied and the type of observation to be made.

Various stains were used, but for nuclear counts iron-alum haematoxylin with an orange G counter stain in clove oil proved to be most satisfactory. Feulgen's (18) reagent was tried on all of the species, but the resulting visibility as a rule was poor. In *Mycena capillaris,* however, it was the only stain which was reliable for nuclear counts, the others giving no differentiation whatever. Each species was a separate problem, but in general if haematoxylin was satisfactory, the other stains were also, and if the haematoxylin did not take readily, the others were equally unsatisfactory.

TAXONOMIC DATA

A careful taxonomic study has been made of all of the species in the genus which the writer has collected. In this study fruit-bodies with four-spored basidia were considered typical. Two-spored forms were referred to a species only when the fruiting bodies resembled those bearing the four-spored basidia in every respect except in the number of spores born on a basidium and in the size and shape of the spores. Fruiting bodies with only four-spored basidia will be referred to hereafter as "typical forms," while those showing variation, being either two-, three- and four-spored on a single pileus, two- and three-spored, or simply two-spored, will be referred to as "variant forms." During the course of the investigation a total of ninety-five typical and variant forms were collected in Michigan; twenty-seven of these are variants. Fourteen of the variants have been correlated with typical forms, seven determined but not correlated, and six remain unidentified.

The variant forms were mostly two-spored, but in practically all a few three-spored basidia were found. In *Mycena citrinomarginata, M. dissiliens, M. leptocephala,* and *M. polygramma* var. *albida* variant forms were found with two-, three-, and four-spored basidia on a single pileus. In all cases, even on a single pileus, the spores born on two- or three-spored basidia were found to be larger than those born on four-spored basidia. The following table summarizes the change in spore size which was found to

accompany a change in the number of spores produced on a basidium. The spores of some of the species are illustrated on plate 34.

TABLE 1

COMPARISON OF SPORE-SIZE OF VARIANT AND TYPICAL FORMS OF SPECIES OF *Mycena*

Species	Variant Form [4]	Typical Form
M. alcalina	11–12 × 6.5–8 μ	8–10 × 5–6 μ
M. capillaris	11–13 × 5–6 μ	8–10 × 4–5 μ
M. **citrinomarginata*	12–14 × 5–6 μ	9–11 × 5–6 μ
M. clavicularis	10–12 × 5–6 μ	8–10 × 4–5 μ
M. **dissiliens*	7– 8 × 7 μ	7–8 × 5–6 μ
M. epipterygia	10–12 × 6–8 μ	9–11 × 6–7 μ
M. graveolens	8–10 × 4.5–5 μ	7–8 × 4 μ
M. immaculata	8–10 × 2.7–3 μ	7–8 × 2.5–3 μ
M. lactea	7.5–8.5 × 6–7.5 μ	7–8 × 3–4 μ
M. **leptocephala*	11–14 × 5–6.5 μ	7–10 × 4–5 μ
M. metata	8–9 × 4–5 μ	7–8 × 3–4 μ
M. **polygramma* var. *albida*	9–11 × 6–7 μ	8–10 × 5–6.5 μ
M. roseipallens	7–8 × 5–6 μ	6–7 × 3–4 μ
M. supina	8–11 × 7–8 μ	7–8 μ
M. viscosa	9–11 × 6.5–8 μ	8–9 × 5–6 μ

[4] The forms commonly three-spored are marked with an asterisk.

In *Mycena dissiliens* and *M. polygramma* var. *albida* there is little difference in size, and the increase is so distributed that the shape of the spore in both species is practically unaltered. In *Mycena citrinomarginata* there is a substantial increase in the length but not in width, thus accentuating the somewhat cylindrical shape of the spore. The species *M. alcalina, M. capillaris, M. clavicularis, M. graveolens, M. metata,* and *M. viscosa* all show an increase in spore size, but scarcely any change in shape. In *Mycena roseipallens, M. lactea,* and *M. supina* a change in shape has accompanied the increase in size. In the first two the change is from a more or less ellipsoid spore to one which is subglobose. In *Mycena supina* the change is from a globose spore to one which is broadly ovate. In all of the forms studied the change was constant within a species.

CYTOLOGICAL AND CULTURAL DATA

The typical forms: Nine four-spored species have been studied cytologically. These show the usual nuclear history through the

meiotic divisions, and the reorganization of the four daughter nuclei. The young basidium is characterized by the presence of two nuceli which fuse to form a large fusion nucleus. The fusion nucleus divides in the upper part of the basidium, and two reorganized daughter nuclei are regularly found. Each of these divides once, and the resulting four nuclei move toward the base of the basidium. After the spores have started to form, the four nuclei elongate and migrate upward, each into a sterigma. However, instead of migrating on into the spore as is usually the case in other agarics, each nucleus divides, one of the daughter nuclei migrating into the spore and the other eventually returning into the upper part of the basidium. Distinct division figures (PLATE 35, FIGS. 9, 27) have been found in *M. Atkinsoni* and *M. viscosa.* As a result of this division, each spore receives one nucleus and four nuclei remain in the collapsing basidium (PLATE 35, FIG. 10). These residual nuclei are constantly present in the old basidia of all of the above species except *Mycena murina,* and while third division figures have not actually been seen for all, the presence of residual nuclei in the collapsing basidia is here considered as evidence that such a division occurred.

In *Mycena murina* a striking variation from this behavior is found. In this species the third division is irregular but characteristic, and all of the daughter nuclei migrate, two into each spore. While third division figures are occasionally found with their axes arranged parallel to the longitudinal axis of the basidium (PLATE 35, FIG. 21), they are usually arranged transversely (PLATE 35, FIG. 20). As is shown (PLATE 35, FIG. 20), each of the two poles of the uppermost division figure lies beneath a sterigma. Various other stages of the division and migration of the nuclei are shown (PLATE 35, FIG. 29, 25, 26, 30, 31). All of the old basidia in this species were completely empty.

It is evident that the arrangement of the spindles in the third division may possibly have an important bearing upon the genetical identity of the nuclei in the four spores. If the heterotypic division is either the first or second division in the basidium, and if the spindles of the third division are in the sterigmata and longitudinal, one would expect two daughter nuceli, identical genetically, to migrate into a spore. On the other hand, if the spindles of the

third division are arranged transversely and daughter nuclei migrated into different sterigmata, the pairs of nuclei seen in figure 26, plate 35 might be of different genetical constitution. Since both arrangements of the third division spindles have been found in the basidia of a single pileus, this species is particularly interesting from a genetical point of view.

The variant forms (usually two-spored): The variant forms studied fall into two types; those in which the young basidium contains two primary nuclei which fuse, giving rise to a fusion nucleus, and those forms with a single nucleus in each young basidium.

The "*Mycena metata*" group: The first group of species contains *M. metata, M. clavicularis, M. graveolens, M. immaculata, M. margaritispora, M. mirata* and *M. viscosa.* The species in this group are characterized by a fusion of two primary nuclei eventually followed by three divisions in the basidium. Since this type of nuclear behavior, with the exception of the third division, is well described in the literature, it will be outlined here briefly for only one species, *Mycena metata.* The cells of the subhymenium are typically binucleate, but occasionally the larger cells are multinucleate. As the young basidium elongates the two nuclei come to lie side by side in the midportion and usually fuse before the basidium has reached its full length. Apparently the fusion takes place rather slowly. The nuclei gradually lose their outlines at the point of contact and are then distinguishable as two granular masses with a darkly staining nucleolus in each.

As soon as the fusion is complete the spireme threads appear as rather coarse, heavily staining strands (PLATE 35, FIG. 40). The fusion nucelus is 3–4 μ in diameter and one or two nucleoli can be seen within it. It gradually increases in size and moves toward the apex of the basidium. Instead of migrating directly into the apex, it comes to rest some distance below it. Judging by the frequency of such figures in all of the preparations, this "resting stage" is one of long duration. Just prior to dividing the nucleus moves farther up toward the apex and the first division follows (PLATE 35, FIG. 41). The details of the division were not worked out, but the spindle is formed within the old nuclear membrane, and a centrosome is visible at each pole. A side view

of a metaphase plate shows that at this stage the whole figure is still within the space previously occupied by the fusion nucleus. However, during the anaphase the centrosomes move back toward the walls of the basidium and at the telophase the two masses of chromatin are often found appressed against the opposite walls. The fate of the nucleoli during the division is uncertain, but they seem to be extruded into the cytoplasm where they are then indistinguishable from other granules.

The figures of the second division are very minute, but metaphase and telophase stages are frequent (PLATE 36, FIG. 1, 2). The four reorganized daughter nuclei are very small at first. They soon increase to 1.5–2 μ, develop a delicate characteristic reticulum, and migrate toward the base of the basidium. This migration carries the nuclei into the more or less constricted portion of the basidium. Just before they come to rest near the base, the sterigmata begin to develop, first as two obtuse humps, but soon elongating and assuming their characteristic narrowly conical form.

When the spores are quite large, the four nuclei situated in the lower portion of the basidium move toward the sterigmata and become elongated or beaked. The behavior of the four nuclei from the time they first become beaked to the time when the two spores are mature seems to be rather variable. As is shown in plate 36, fig. 3, the spores begin to form before the nuclei elongate. The two nuclei which lie uppermost in the basidium migrate first. Figures can be found showing the first nucleus, after it has migrated to the apex of the spore, either in a resting condition or dividing. Figures of daughter nuclei can also be found indicating that a division has been completed. The second set of nuclei may enter the spores and divide immediately (PLATE 36, FIG. 4) leaving the basidium empty, or they may divide while in the sterigmata (PLATE 36, FIG. 5). Only one of the daughter nuclei of this division then passes into the spore. The divisions in the spores and sterigmata are rather haphazard. In plate 36, fig. 4, the nucleus nearest the sterigmata is shown dividing while the apical nucleus is in a resting condition. In plate 36, fig. 5, the nucleus in one spore and those in the sterigma are shown dividing simultaneously. In plate 36, fig. 6, two nuclei in one spore are shown in the resting condition while the one nucelus in the other spore and also the nucleus just

entering the latter, are shown in practically the same stage of division. One basidium showing two resting nuclei in one spore, and two dividing nuclei in the other was also found (PLATE 36, FIG. 7).

In *Mycena margaritispora* the situation seems to be similar to that found in *Mycena metata,* the third division figures occurring rather haphazardly in both spores and sterigmata. The manner of origin of the four residual nuclei (PLATE 36, FIG. 20) has not been determined. As shown (PLATE 36, FIG. 29), in *Mycena viscosa* all four nuclei may migrate, two into each spore, and then divide simultaneously, the basidium showing no residual nuclei. However, old basidia with residual nuceli are found in this species also. In *Mycena graveolens* two residual nuclei are commonly present, occasionally four, and very seldom none at all.

The material of *M. clavicularis* and *M. immaculata* was not favorable for studying the later stages.

The "*Mycena megaspora*" group: The species of this, the second group of variant forms, are characterized by the lack of a fusion of nuclei in the basidium, the single original nucleus eventually giving rise to four daughter nuceli. Twelve species, namely *M. megaspora, M. alcalina, M. capillaris, M. cholea, M. citrinomarginata, M. dissiliens, M. lasiosperma, M. leptocephala, M. polygramma* var. *albida, M. roseipallens, M. rubromarginata* var. *Laricis,* and *M. vitilis* have been found to belong to this type. All of these are similar to the two-spored form of *Camarophyllus virgineus* studied by Bauch (1) in that the subhymenial cells and young basidia each contain a single nucleus. Because there is no fusion of nuclei in the basidium, and because the same number of chromosomes was found in the nuclei of both two- and four-spored forms, Bauch has considered the two-spored form of *C. virgineus* to be parthenogenetic. In *Mycena alcalina* which has received considerable study, the writer's observations indicate that the chromosome number, as obtained from division figures in the basidia, is apparently the same in the two-, and the four-spored forms. Since this corresponds to Bauch's findings, the writer is classifying all the forms in the "*Mycena megaspora*" group as haploid parthenogenetic forms. In the treatment which follows, *M. citrinomarginata, M. dissiliens, M. leptocephala* and *M. polygramma* var. *albida* are considered separately because they illus-

trate the nuclear behavior in parthenogenetic forms producing two, three, or four spores on a basidium.

Since *Mycena megaspora* has been studied culturally and cytologically, it will be discussed here is some detail. This species has been cultured from transfers of tissue from the pileus, groups of spores sprayed on agar plates, and from single spores isolated by the spray method (**9**). No clamp connections were observed in any of the cultures. The fungus grows rather slowly covering the agar in an ordinary petri dish in six to eight weeks. A 2.5 per cent malt extract agar was the most favorable medium tried, and the optimum temperature was found to be between 23 and 25 degrees centigrade. Tissue cultures grew more rapidly than the single spore cultures, but there was no difference in the type of growth produced. The hyphae grew very closely together in a compact mat along the surface of the agar and produced a fluffy, white aerial growth. The medium was penetrated to a depth of one or two millimeters by a rather sparse growth.

Fifty single spores were isolated during the course of the work, and mating experiments were carried out in an attempt to obtain diploidization. Mycelia from spores from fruit-bodies collected in different localities were used in the mating experiments, but no clamp connections were produced. Since certain Basidiomycetes (**20**) are known to possess the dikaryophase without showing clamp connections on the mycelium, an attempt was made to study the mycelium obtained from tissue cultures and from single spores cytologically. Due to the slow and compact manner of growth the method recommended by Sass (**21**) failed to give any results, and it was necessary to kill and embed mycelium grown on agar plates. Sections were cut 12 to 25 μ thick and stained with iron-alum haematoxylin or safranin and light green. While this method has obvious disadvantages, it was found to be usable. As a check, the mycelium of *Collybia clusilis* Fries, a species which produces clamp connections abundantly, was carried through the same process. Slides of *C. clusilis* showed two nuclei in each cell and abundant clamp connections while the slides of *Mycena megaspora* showed one nucleus in each cell, or occasionally two or three in very large hyphae.

In the fruit-body the cells of the stipe were found to contain

from one to six or more nuclei depending on the size of the cell. The tissue of the stipe in this species is composed of large, straight, tubular cells as well as irregular hyphae of smaller diameter which lie intertwined among the larger elements. The individual cells of the irregular hyphae were usually found with one nucleus, while the enlarged, parallel cells always contained two or more. The latter condition was also found in the enlarged cells of the pileus trama, but as a rule the cells of the gill-trama and especially those of the subhymenium were found to be uninucleate.

The single nucleus in the young basidium enlarges, keeping pace with the developing basidium (PLATE 37, FIG. 3), and finally can be found as a large and conspicuous structure just below the apex. In this stage it resembles the large fusion nucleus found in the species previously discussed. The spireme threads, however, are not coarse and their behavior prior to the single division at the apex of the basidium is not characteristic of meiosis. The large nucleus may or may not migrate up to the apex of the basidium to divide. The division figure is rather large in this species and the centrosomes are conspicuous. Here as in *Mycena metata,* the figure is formed within the old nuclear membrane. The daughter nuceli are at first very small, but enlarge rapidly as they migrate toward the base of the basidium.

The daughter nuclei stop when they have passed slightly back of the midpoint of the basidium, and at this time two sterigmata begin to develop. As the spores increase in size, the nuclei migrate upward, and as the latter approach the sterigmata they begin to elongate, assuming the beaked condition. The centrosome is visible as a darkly staining granule at the apex of the beak. One nucleus migrates into each spore. While passing through the sterigmata the fine chromatin network becomes drawn out into long strands, but is always visible in well fixed material. The two nuclei do not always migrate simultaneously but figures have been found with a nucleus present in each sterigma. As a rule, after the migration downward, one nucleus comes to rest a little below the other and remains a little behind on its migration into the spore.

Although no clearly defined division figures were found in the sterigmata, the presence of residual nuclei in the collapsing basidia

indicate that a division must have occurred. Such nuclei were usually present in *Mycena alcalina, M. cholea, M. rubromarginata,* var. *Laricis, M. roseipallens,* and *M. vitilis.* They were uncommon in *Mycena megaspora* and *M. capillaris,* and absent in *M. lasiosperma.* In *M. cholea* (PLATE 37, FIG. 16) a basidium is shown with both spores attached, each with a nucleus, and two remaining residual nuclei in the basidium. Since no basidia with four nuclei present before migration have been found in this species, the situation shown in figure 16 must have been brought about by a division when the nuclei were migrating into the spores. In *M. lasiosperma* (PLATE 37, FIG. 26) the nucleus is shown dividing after it has migrated into the spore. The migration figures (PLATE 37, FIGS. 19, 34) of *M. lasiosperma* and *M. roseipallens* are interesting. In these species the nuclear reticulum does not show any affinity for the stain and appears to be made up of a fine, hyaline, granular network. The nucleolus appears as a large opaque structure which holds the stain tenaciously, and may be mistaken for the nucleus in poorly stained preparations. During the migration of the nucleus the nucleolus becomes beaked in a characteristic manner within the nucleus and migrates within the latter into the spore. In a majority of the species studied the reticulum is characterized by the presence of darkly staining chromatin granules, and the nucleolus does not migrate into the spore as a distinct unit.

The parthenogenetic forms producing two-, three- and four-spored basidia: Since *Mycena polygramma* var. *albida* lends itself well to cytological investigation, it will be treated in detail here. The behavior of the nuclei corresponds exactly with that of the haploid parthenogenetic forms previously discussed until they are on their way into the spores. The sterigmata begin to form at about the time the two daughter nuclei cease their downward migration. As the sterigmata develop the nuclei begin to move upward toward the apex, but, after moving a short distance, each develops a long beak which stretches out, in some instances at least, almost into a filament. The apex of the beak may come to rest somewhere near the base of a sterigma or may actually enter one. Apparently the remainder of the nucleus is now drawn upward, and the beak becomes shorter and shorter. At this time

two, three, or four sterigmata are usually well developed and a spore is forming on each. When only two sterigmata are formed the nuclei do not necessarily come to rest at the apex of the basidium, but may migrate into the spore in the usual manner. Occasional residual nuclei in collapsing two-spored basidia, however, indicate that a division sometimes takes place during the migration. If three sterigmata are formed, either one of two things may happen. Occasionally the two nuclei migrate, each into a spore, leaving the third spore without a nucleus. Rather conclusive evidence of this type of behavior was found (PLATE 38, FIG. 21). In most cases, however, the three-spored basidia produce spores with one nucleus in each. This is brought about in the following manner. One nucleus rounds up near the base of a sterigma and undergoes a division, one pole lying just below one sterigma and the other pole near or under a second. As the division progresses the poles of the figure are drawn up into the sterigmata, and a U-shaped figure results (PLATE 38, FIG. 17, 18). The daughter nuclei reorganize, one in each spore. The second nucleus may also round up, but was never seen to divide in a three-spored basidium. It usually migrates just before or with the daughter nuclei resulting from the division of the first nucleus.

If the basidium produces four sterigmata, three things may happen. First, each spore may receive one nucleus. This is commonly the case and is brought about in the following manner. Each of the two nuclei which have migrated from the base of the basidium round up somewhere near the base of a sterigma. In this position each nucleus undergoes a division, the poles coming to lie under the sterigmata, and during the anaphase the unorganized nuclei begin to migrate, forming characteristic U-shaped figures. The daughter nuclei reorganize as soon as they have moved into their respective spores. The U-shaped figures were always found in pairs and there was no indication that two nuclei ever migrated into a single spore.

Secondly, one nucleus may migrate directly into one spore while the other divides in the manner described above. Thus three spores would be found with nuclei while the fourth would be without one. One basidium (PLATE 38, FIG. 29) was found indicating this.

Lastly, the two nuclei at the base of the basidium may migrate directly, one into each of two spores, thus leaving two spores without nuclei. No evidence for this type of behavior was found.

Mycena leptocephala, M. citrinomarginata, and *M. dissiliens* all exhibit this same general type of behavior. The very minute nuclei in *M. dissiliens* make it a rather unfavorable subject for study, but typical stages were found. The spores become detached from the sterigmata of *M. citrinomarginata,* and *M. leptocephala* very easily, thus making it impossible to follow the migration of the nuclei. Collection 32–597 of *M. citrinomarginata* was found to be parthenogenetic and predominantly four-spored. The nuclear behavior was similar to that described above.

GENERAL CONSIDERATIONS

In the present investigation it has been shown that in nature fruiting bodies with two-spored basidia occur along with fruit-bodies bearing four-spored basidia, and that the only differences between the two are the number of spores born on a basidium and a correlated change in spore size. In certain species it has been found that these differences occur in a single pileus as well as in pilei from different localities, and at times a cytological study must be made to determine whether a form is typical or not. Such forms are clearly segregates of four-spored species. In other species the two-spored forms are more infrequent and are separated geographically from the normal form. The close similarity in all of the characters except those mentioned above, however, indicates that these also are segregates from typical forms. Thus the majority of the two-spored forms collected can reasonably be considered as segregates of the typical forms, and it is reasonable to assume that in such isolated two-spored species as *Mycena margaritispora* and *M. lasiosperma* we may be dealing with the segregated forms of otherwise unknown species. It is possible that some of these isolated two-spored species may be stable forms which have persisted while the ancestral forms have been lost in the course of evolution. It is probable that a similar relationship exists for the two-spored forms in other genera of the Agaricaceae.

As has been previously stated, the two spored character has been

used as a diagnostic character for the separation of taxonomic units. Sass (21) has suggested that the term "forma" be used "in clear cut cases." Such a designation, however, would, besides being somewhat cumbersome, give undue emphasis to the two-spored condition. For instance, if a species was found to have two-spored forms of both the "*M. metata*" and "*M. megaspora*" type, they would all be classed as *forma bispora* in spite of the difference in the behavior of the nuclei in their basidia. The writer prefers to simply speak of the segregates as forms without assigning a name to them. By so doing no implication is made concerning the relationships of one form to the other except that both are segregates of the species to which they have been assigned. In species like *M. polygramma* var. *albida* or *M. citrinomarginata* where, without a cytological examination, it is impossible to ascertain with certainty whether or not one has parthenogenetic or normal fruiting bodies, a form name would serve no purpose.

The two-spored condition also results in a change in spore size which is of importance in the differentiation of taxonomic units. The writer's observations cover a sufficiently large number of species to give a fair idea of the frequency with which the change to the two-, or three-spored condition is accompanied by an increase in spore size, the amount of this change, and its bearing on the value of spore size as a character of major taxonomic importance.

As has been shown in table 1, the larger spore size was a constant feature of the variant forms, but it seems to depend upon the species whether the shape is similar or different. Thus it is possible to predict that in the genus *Mycena,* as two-spored forms are found, the spores will measure larger than those of the typical forms. No prediction is justified, however, concerning the shape of the spore. Since this situation exists, the writer feels that the differences in the basidia and spores of variant forms, particularly in the genus *Mycena,* should not be used as a basis for establishing species. In typical forms, however, spore size and shape are remarkably constant and useful in separating species within the genus.

Since the basidia of two- and four-spored forms seem to be

approximately the same size, the question arises as to whether or not within a species, the volume of the two spores produced on a single basidium is equal to the volume of the four spores also from a single basidium. The shape of the spores of two species is such that rough approximations of their volumes can be easily made. In *M. capillaris* and *M. citrinomarginata* the spores are roughly cylindrical in shape. The following approximations may be made:

M. capillaris (4-spored) 143.3 cu. microns per spore
573 total volume of 4 spores.
M. capillaris (2-spored) 285 cu. microns per spore
570 total volume of 2 spores.
M. citrinomarginata (4-spored) 196.6 cu. microns per spore
786 cu. microns total volume of 4 spores.
M. citrinomarginata (3-spored) 255.2 cu. microns per spore
765 cu. microns total volume of 4 spores.

While only approximations, these figures indicate that there is little if any change in the volume of material used in the formation of the spores in the correlated forms, and that the difference in spore-size may possibly be explained on the basis of the amount of material available for spore formation. One would expect the above ratio to be rather constant in forms producing both two- and four-spored basidia on a single pileus. The variation in size of basidia and spores of geographical races might be sufficient to overbalance this difference if the two-spored form from one locality is compared with a four-spored form from a different locality.

In the species of *Mycena* investigated in this study the basidia were always emptied of protoplasm before the spores were shed. If this does not happen, however, it is doubtful if there would be an increase in spore size to the extent described above. In other genera the basidia of the variant forms are not always emptied of protoplasm as completely as in *Mycena* and in such cases it is not likely that an equal increase in spore size would be found in the variant forms. *Naucoria semiorbicularis* f. *bispora* Sass (21) seems to be an example.

CYTOLOGICAL CONSIDERATIONS

The typical forms: The nuclear behavior in eight of the four-spored species agrees with that found in *M. galericulata* as described by Maire (17) except that the spores are usually uninucleate. The occasional binucleate spores which were found may have obtained their nuclei either by the division of the nucleus after entering the spore, or by the migration into the spore of the daughter nuclei resulting from a third division.

Wager (22) figured two reorganized nuclei resulting from the first division in *Mycena galericulata.* Wakayama (23) in his studies of a large number of agarics in various genera found that in *Mycena haematopoda* the daughter nuclei of the first division always reorganize before undergoing the second. In species of other genera, he found no period of interkinesis. All of the forms studied by the writer exhibiting meiosis also show a similar short period of interkinesis between the first and second divisions.

Evidence of the regular occurrence of a third division in the basidium has been found in all of the typically four-spored species investigated and may well be considered typical for the genus. It has been shown that this division is postponed until the nuclei are starting to migrate or are actually migrating into the spores. It is thus definitely removed from the meiotic divisions in time as well as location. In the species of *Craterellus* and *Clavaria* which have been studied (8) the third division may either follow soon after the first two, and before the nuclei have begun to migrate, or take place in the basidium after the spores are partly formed.

Two-spored forms of the *Mycena metata* group: The third division which was found to be characteristic of the four-spored forms is also present in the group of two-spored forms characterized by *Mycena metata.* In this group we find a situation essentially similar to that found by Sass (21) in the two-spored form of *Coprinus ephemerus.* Two nuclei regularly migrate into each spore. Because attempts to germinate the spores in this group of species have been unsatisfactory, it has been impossible to determine whether they are "heterothallic" or "homothallic." Since their nuclear history is essentially like that of the two-spored form of *C. ephemerus,* however, one would expect them to be "homothallic."

In this group the fusion nucleus gives rise to four daughter nuclei. One of the daughter nuclei migrates into each spore where it divides. The remaining two nuclei may divide in the sterigmata or after entering their respective spores. If the division takes place before entrance, one would expect to find residual nuclei in the basidium. If it takes place after the nucleus enters the spore, the basidium should be empty. Division figures have been found in the spores and sterigmata of most of the species in this group. Both empty basidia and those with residual nuclei have also been found.

Since both Sass and Buhr have interpreted the residual nuclei which they observed in the collapsing basidia of the forms they studied as remains of two of the original four nuclei produced by the two meiotic divisions, their descriptions and figures should be reconsidered in the light of the above facts. In the two species of *Mycena* which Buhr studied, the residual nuclei could have originated in either manner. The basidia shown in his figures do not have the spores attached. In *Nolanea cetrata,* however, his illustrations are convincing enough. The attached spores (FIG. 11) each have a nucleus and two have remained in the basidium. In *Psalliota campestris* (FIG. 24) the residual nuclei show their usual structure as do those of *Pholiota erebia* (FIG. 4). Sass (21), plate 66, figures 60 and 61 of *Naucoria semiorbicularis,* illustrates the residual nuclei as possessing their normal structure, while on plate 67, fig. 73 the one residual nucleus shown for *Galera tenera* is opaque.

In all of the writer's slides the residual nuclei stained very darkly, similar to the telophases of the earlier divisions, and were never found to reorganize sufficiently to show either a reticulum or nucleolus. Buhr's (4) figures 36 and 37 of *Mycena debilis* certainly illustrate a condition closely approaching this. It is quite possible that the residual nuclei originate differently in different species or genera. Certainly if two of the original four nuclei disintegrate, one would expect to find early stages in which the structure of the nucleus was still visible. If, on the other hand, the nuclei left in the basidium by the third division degenerate without reorganizing, no such early stages could be expected.

The omission of a division in the parthenogenetic forms studied

by the writer is interesting because it furnishes still more evidence in support of the statement that three divisions are characteristic in the basidia of this genus. In both the typical forms and those of the *M. metata* type two divisions in the apex of the basidium are characteristic before the sterigmata begin to form. In the parthenogenetic forms only one such division occurs. Thus it is reasonable to assume that the reduction divisions are omitted and in their place a single mitosis occurs. This is apparently the same situation which Bauch (1) disscovered in *Camarophyllus virgineus.* In the two-spored form of that species Bauch found that one of the two nuclei resulting from the single division at the apex of the basidium migrated into each spore. In the parthenogenetic forms studied by the writer two divisions were characteristic, the second, however, corresponded closely in both time and irregularity of occurrence to the third division found in the typical forms and in the variants of the *M. metata* type. It is no doubt homologous with it.

The manner in which the last division functions in the distribution of nuclei to the various spores of the forms producing two, three and four sterigmata is interesting. As a result of it, it is possible to have four-spored forms in the genus *Mycena* which are indistinguishable unless the material is examined cytologically. Such forms have been collected. No particular significance can be attached to the spores which do not receive nuclei, but it would be interesting to determine their approximate percentage and whether or not they are discharged from the basidium in the usual way. Since two-spored forms of both cytological types are common in a single genus it is apparent that the nuclear history is not necessarily concerned with the number of sterigmata produced on a basidium. In the *M. metata* type the behavior of the nuclei is typical, but the basidia are constantly two-spored. In the *M. megaspora* type the nuclear behavior varies but the number of sterigmata may be four. That the two-spored condition is a constant genetical character in the forms of the *M. metata* type is borne out to some extent at least by the fact that the forms of this group can be collected year after year in the same as well as in widely scattered localities under a variety of weather conditions. The parthenogenetic series, however, does not present a clear cut

case because of the numerous specimens producing two, three, and four spores on different basidia in a single pileus.

The results of the present investigation when considered in the light of previously known facts indicate that in a closely related group as well as in a group of distantly related forms, the same types of variation in the nuclear behavior are encountered. Buhr (4), summarizing the information concerning two-spored forms in the Hymenomycetes, lists sixteen species having a normal fusion with the customary two divisions following (except in the species of *Clavaria* and *Craterellus* which have a third). In contrast to this, only two are listed definitely as being parthenogenetic. He includes two species of *Mycena* in the first group and one in the second. The results of this investigation add seven species of *Mycena* to the former group, twelve to the latter, and prove conclusively that the parthenogenetic type of variant is common in the genus.

The forms and species showing a reduction in the number of spores born on the basidium presents an interesting series in the Hymenomycetes. Species of Clavariaceae and Cantharellae are known to have basidia bearing more than four spores and also to have two-spored forms. In the Agaricaceae, four sterigmata are usually formed. Parthenogenetic four-spored forms as well as those varying between the two- and four-spored condition are known in *Mycena*. In *Galera*, Bresadola (3) has figured a form of *G. silignea* with monosporous basidia. In *Naucoria lenticeps* Peck, fruiting bodies bearing basidia with one, two, three or four sterigmata have been found by the writer. Thus within a single species or in the group as a whole variations of from one to four spores are known to occur.

The presence of a third division in most of the basidia of typical and variant forms of *Mycena* supports the hypothesis that the species of this genus are rather closely related to primitive types which are characterized by three successive nuclear divisions in the basidium. The variations found indicate that the character is in a transitional state.

SUMMARY

1. During the course of the present investigation sixty-five typical and twenty-seven variant forms were collected.

2. One species *Mycena cholea* and one variety *Mycena rubromarginata* var. *Laricis* are described as new.

3. Of the twenty-seven variant forms collected, fourteen have been correlated with typical forms.

4. An increase in spore size has been found to accompany the change to the two- or three-spored condition. A change in spore shape may or may not accompany the change in the number of spores born on a basidium.

5. The writer believes that the variations in spore size and shape noted for the variant forms should not be used as basic distinctions in describing new species.

6. Nine typically four-spored forms have been studied cytologically. In eight, *M. Atkinsoni, M. alcalina, M. hemisphaerica, M. haematopoda, M. leptocephala, M. sanguinolenta, M. stannea* and *M. viscosa* the nuclear history is characterized by a fusion of two primary nuclei followed by two meiotic divisions, and a third division before the nuclei migrate into the spores. Four nuclei usually degenerate in each basidium. In *Mycena murina* there is a similar third division, but each spore receives two nuclei.

7. Of the variant forms studied, *M. metata, M. clavicularis, M. graveolens, M. immaculata, M. margaritispora, M. mirata,* and *M. viscosa* all possess two primary nuclei which fuse. Three divisions were found to be characteristic, but the third was rather haphazard, occurring either in the spores or basidia. *M. megaspora, M. alcalina, M. capillaris, M. cholea, M. citrinomarginata, M. dissiliens, M. lasiosperma, M. leptocephala, M. polygramma* var. *albida, M. roseipallens, M. rubromarginata* var. *Laricis,* and *M. vitilis* were found to have a single nucleus in the young basidium and are considered to be parthenogenetic. Instead of the usual three divisions found in the other forms in this genus, the parthenogenetic forms were characterized by two, the last of which is apparently homologous with the third division found in the other types.

8. Four of the parthenogenetic forms, *M. citrinomarginata, M. dissiliens, M. leptocephala* and *M. polygramma* var. *albida* were found to produce two, three, or four spores on different basidia of a single pileus. In these forms the transverse arrangement of the spindles in the second division was found to form a mechanism whereby a nucleus was usually distributed to each spore. The

process, however, was found to be rather variable and spores without nuclei were found. The latter condition may be brought about by a nucleus failing to undergo the last division before migration, or possibly by the longitudinal instead of the transverse arrangement of the second division spindle.

9. Since both the *M. metata* and *M. megaspora* types of variants are common in *Mycena*, it seems improbable that the nuclear behavior in the basidium is in any way connected with the number of spores produced on a basidium.

EXPLANATION OF PLATES

PLATE 34

The drawings were made with the aid of a camera lucida using a Bausch & Lomb 3 mm. N. A. 0.85 objective and a 25 × ocular. As reproduced, the magnification is approximately 830 ×.

Fig. 1, Spores of four-spored form of *Mycena epipterygia*.
Fig. 2, Spores of two-spored form of *Mycena epipterygia*.
Fig. 3, Spores of two-spored form of *Mycena alcalina*.
Fig. 7, Spores of four-spored form of *Mycena alcalina*.
Fig. 4, Spores of two-spored form of *Mycena capillaris*.
Fig. 5, Spores of four-spored form of *Mycena capillaris*.
Fig. 6, Spores of two-spored form of *Mycena roseipallens*.
Fig. 9, Spores of four-spored form of *Mycena roseipallens*.
Fig. 8, Spores of four-spored form of *Mycena viscosa*.
Fig. 11, Spores of two-spored form of *Mycena viscosa*.
Fig. 10, Spores of two-spored form of *Mycena lactea*.
Fig. 13, Spores of four-spored form of *Mycena lactea*.
Fig. 12, Spores of four-spored form of *Mycena immaculata*.
Fig. 14, Spores of two-spored form of *Mycena immaculata*.
Fig. 15, Spores of four-spored form of *Mycena metata*.
Fig. 16, Spores of two-spored form of *Mycena metata*.
Fig. 17, Spores of three-spored form of *Mycena citrinomarginata*.
Fig. 18, Spores of four-spored form of *Mycena citrinomarginata*.
Fig. 19, Spores of two-spored form of *Mycena mirata*.

PLATES 35–38 INC.

The drawings were made with the aid of a camera lucida using a Zeiss 2 mm. N. A. 1.3 apochromatic objective and a 20 × compensating ocular. All are reproduced at a magnification of approximately 1100 ×.

PLATE 35

Fig. 1–10 inc. *Mycena Atkinsoni*. 1, a young basidium showing two primary nuclei; 2, the fusion nucleus; 3, a metaphase stage of the first division; 4, reorganized nuclei after first division; 5, telophase of second division; 6,

four reorganized daughter nuclei; 7, four nuclei near the center of the basidium before the spores have begun to form; 8, four spores, each with a nucleus, and four residual nuclei in the basidium; 9, telophase of third division; 10, old basidium with four residual nuclei.

Fig. 11–26 and 29–32 inc. *Mycena murina.* 11, young basidium with two nuclei; 12, fusion nucleus; 13, first division; 14, two of the four daughter nuclei which have migrated up from near the base of the basidium in preparation for the third division; 15, four daughter nuclei in the upper part of the basidium a short time after the second division; 16, four nuclei in the lower part of the basidium; 17, telophase stages of the third division, the spores were no doubt broken off; 18, two reorganized nuclei after the first division; 19, telophase stages of second division; 20, metaphase stages of third division, spindles arranged transversely; 21, metaphase stages of third division, spindles arranged longitudinally; 22, three spores with one nucleus each and one nucleus in each of the four sterigmata; 23, metaphase figure of third division, spindle arranged transversely; 24, telophase of third division; 25, one nucleus about to enter a spore and a second dividing at the base of the sterigma; 26, two nuclei in each of four sterigmata; 29, three spores each with a nucleus, and a second nucleus about to enter each spore; 30 and 31, the same basidium, each of the four spores contains two nuclei and the basidium is empty; 32, portion of a basidium showing one spore with a nucleus and a second nucleus dividing transversely at the base of the sterigma (32 and 24 are of the same basidium but at different focal planes).

Fig. 27, 28 and 35, *Mycena viscosa,* four-spored. 27, metaphase of third division, the spores have probably been broken off; 28, telophase of third division; 35, three spores with nuclei, four residual nuclei in the basidium.

Fig. 33, 34, 36, 37, 38, *Mycena hemisphaerica.* 33, third division figures; 34, spores with dividing nuclei, the spores had been broken from their attachment; 36, basidium with four spores each with a nucleus, four residual nuclei in the basidium; 37 and 38, four residual nuclei in each of two old basidia.

Fig. 39–43 inc. *Mycena metata,* two-spored. 39, young basidium with two primary nuclei; 40, fusion nucleus; 41, anaphase of first division; 42, telophase of first division; 43, reorganized nuclei from first division.

PLATE 36

Fig. 1–10 and 12, *Mycena metata,* two-spored. 1, metaphase of second division; 2, telophase of second division; 3, a basidium with four nuclei, two spores are shown in an early stage of development; 4, a basidium with no residual nuclei, one nucleus at the base on one spore shown dividing while the nucleus at the apex is in the resting condition, in the other spore the nucleus at the apex is dividing, it can not be definitely determined whether the nucleus at the base of the second spore had just entered or whether it also was dividing; 5, two nuclei in the sterigmata and one in a spore dividing simultaneously; 6, one nucleus dividing in a sterigma and one in the apex of the spore, in the second spore both nuclei are in the resting state; 7, two nuclei dividing in one spore, two resting nuclei in the other, there are no residual nuclei in the basidium; 8, one spore with three nuclei, one spore with one nucleus (in the telophase stage) and two nuclei in the basidium; 9, a spore with

four nuclei; 10, a spore with two nuclei dividing and no residual nuclei in the basidium; 12, a collapsing basidium with two residual nuclei.

Fig. 13–15, *Mycena stannea,* four-spored. 13, four nuclei beaked, the centrosome is at the apex of the beak; 14, daughter nuclei of the third division; 15, a portion of an old basidium showing two spores, the small basidium shows two primary nuclei.

Fig. 11, 16–21 and 23, *Mycena margaritispora.* 11, a spore with four nuclei; 16, a basidium with two primary nuclei; 17, first division, spindle arranged transversely; 18, second division; 19, third division (in spores and sterigmata); 20, four residual nuclei in an old basidium; 21, two residual nuclei in an old basidium; 23, first division, spindle arranged longitudinally.

Fig. 24–27, and 29, *Mycena viscosa,* two-spored. 24, basidium with two primary nuclei; 25, second division, one spindle longitudinal and the other nearly transverse and viewed from the metaphase plate; 26, four nuclei in the lower portion of the basidium; 27, nuclei elongating preparatory to passing through the sterigmata; 29, an empty basidium with two spores each with two nuclei (all of the nuclei are seen to be practically in the same stage of division).

Fig. 22, *Mycena sanguinolenta,* an old basidium showing residual nuclei and two spores each with a nucleus. Two sterigmata were apparently removed in sectioning.

Fig. 30, 31, 38, and 39, *Mycena mirata.* 30, young basidium with two primary nuclei; 31, four nuclei in a basidium at the time the sterigmata are forming; 38 and 39, old basidia, one showing residual nuclei.

Fig. 35 and 36, *Mycena clavicularis,* two-spored. 35, young basidium with primary nuclei; 36, basidium with four nuclei shortly after the second division.

Fig. 37 and 44, *Mycena immaculata,* two-spored. 37, basidium with two primary nuclei; 44, four nuclei at about the time the spores start to form.

Fig. 32, 33, 40–43, *Mycena graveolens,* two-spored. 32, young basidium with two primary nuclei; 33, a spore with four nuclei; 40, basidium with four nuclei; 41, a basidium with two spores attached, each with three nuclei (it is possible that one nucleus divided while in the sterigma and that the other migrated without dividing. The pairs of nuclei at the apex are interpreted as daughter nuclei resulting from a division in the spore); 42, an old basidium with two residual nuclei; 43, an old basidium with four residual nuclei.

PLATE 37

Fig. 1–10 inc. *Mycena megaspora.* 1, young basidium with a single nucleus; 2, young basidium and subhymenial cells with one nucleus each; 3, a developing basidium with its enlarging nucleus; 4, a metaphase stage of the only division which takes place near the apex of the basidium before the sterigmata begin to form; 5, telophase stage; 6, the enlarging basidium with the two reorganized nuclei; 7, an early stage in the formation of the sterigmata; 8, the two nuclei in the basidium have started to migrate, the spores are still very small; 9, a nucleus partly in the spore and partly in the sterigma; 10, one nucleus is seen in each spore, the basidium contains no residual nuclei.

Fig. 11, 12 and 19, *Mycena roseipallens*. 11, the young basidium with its single nucleus; 12, and 19, the nuclei are shown partly elongated, the nucleolus is also shown migrating as a distinct body within the nucleus.

Fig. 13 and 14, *Mycena rubromarginata* var. *Laricis*. 13, young basidium and basal cell each with one nucleus; 14, two nuclei each migrating into its respective spore.

Fig. 16, 17, 23, *Mycena cholea*. 23, young basidium with a single nucleus; 17, basidium with two nuclei at about the time that the spores begin to form; 16, old basidium with two spores attached, each with a nucleus and two residual nuclei in the basidium indicating that a division must have taken place during migration.

Fig. 15, 22, *Mycena capillaris*, two-spored. 15, the nuclei are elongating preparatory to passing through the sterigmata; 22, young basidium with a single nucleus.

Fig. 20, 21, 26, 27, and 34, *Mycena lasiosperma*. 20, young basidium with a single nucleus; 21, division at apex of basidium; 26, spore showing nucleus in telophase stage; 27, portion of a basidium showing how the nucleolus elongates with the nucleus; 34, nuclei elongating preparatory to entering the spores.

Fig. 18, 24, *Mycena vitilis*. 18, basidium with spore beginning to form; 24, young basidium with a single nucleus.

Fig. 28, 30, 31, 36–38, *Mycena leptocephala*, parthenogenetic form. 28, young basidia and subhymenial cell showing a single nucleus each; 30, a basidium with two sterigmata beginning to form and two nuclei slightly elongated; 31, nucleus dividing at base of sterigmata; 36, young basidium with a single nucleus; 37, the enlarged nucleus prior to dividing; 38, four sterigmata forming on one basidium and only two nuclei in the basidium.

Fig. 35, 42, 43, *Mycena citrinomarginata*, parthenogenetic form. 35, nuclear division at the base of the sterigmata; 42, nuclear division at the apex of the basidium before the spores have begun to form; 43, a basidium with two nuclei and two sterigmata.

Fig. 32, 33, 39, 40, 41, *Mycena dissiliens*, parthenogenetic form. 32, young basidium and basal cell each with a single nucleus; 33, the single division at the apex of the basidium; 39, the two daughter nuclei after they have moved downward; 40, the nuclei near the base of the basidium becoming beaked (note the three sterigmata which are nearly formed); 41, telophase of the division at the base of the sterigma.

PLATE 38

Fig. 1–29, *Mycena polygramma* var. *albida*, parthenogenetic form. All of the figures were drawn from the basidia of a single pileus. 1–7, illustrate the usual type of development in parthenogenetic forms; 8–10, illustrate basidia each with two nuclei, but two, three, and four sterigmata; 11–13, show nuclear elongation in two-spored basidia; 14, the two nuclei have assumed a position which indicates that migration has ceased temporarily (the stage shown in fig. 11 precedes this one); 15–18, portions of basidia showing the division at the bases of the sterigmata; 19–20, nuclear migration in two-spored basidia; 21, a basidium which produced three spores, but in which

both nuclei migrated without dividing thus one spore did not receive a nucleus; 22, a basidium showing two nearly mature spores and one partly developed, the origin of the single nucleus in the basidium can not be definitely determined; 23–24, three-spored basidia, each spore receiving a nucleus; 25, a three-spored basidium; each spore with a nucleus; 26, two nuclei in a basidium and four spores forming; 27, each nucleus divides, and thus each spore receives a nucleus; 28, a basidium with four mature spores, each with a nucleus; 29, a four-spored basidium in which only three spores received nuclei.

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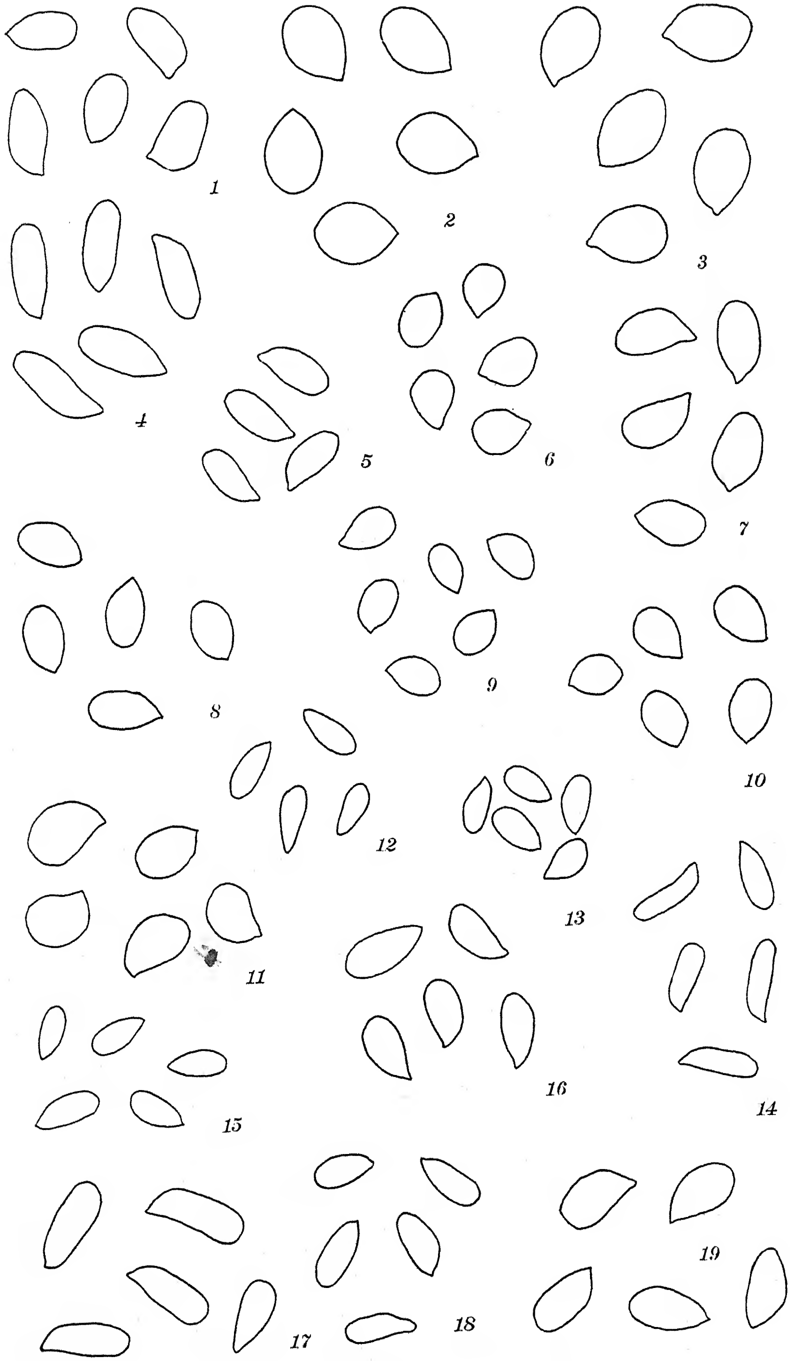

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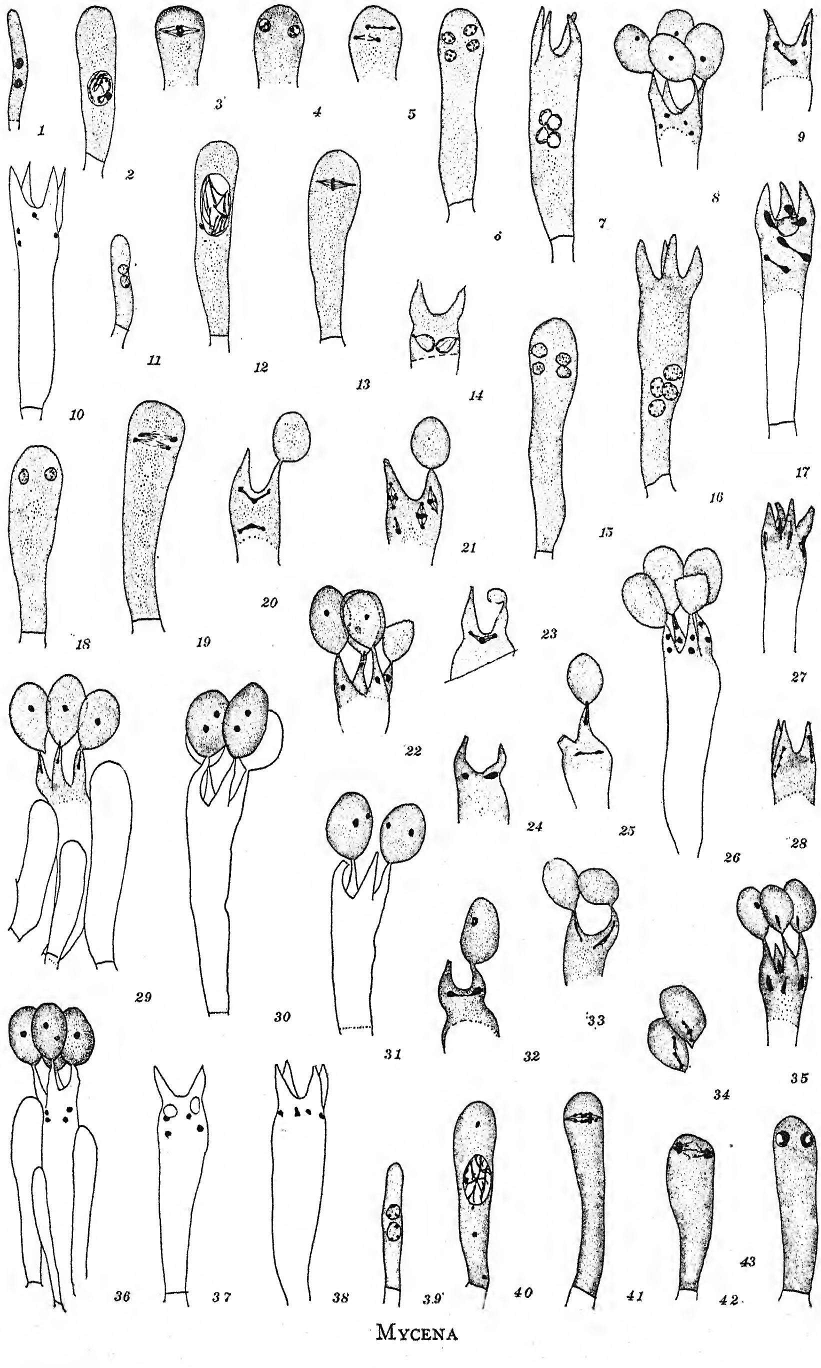
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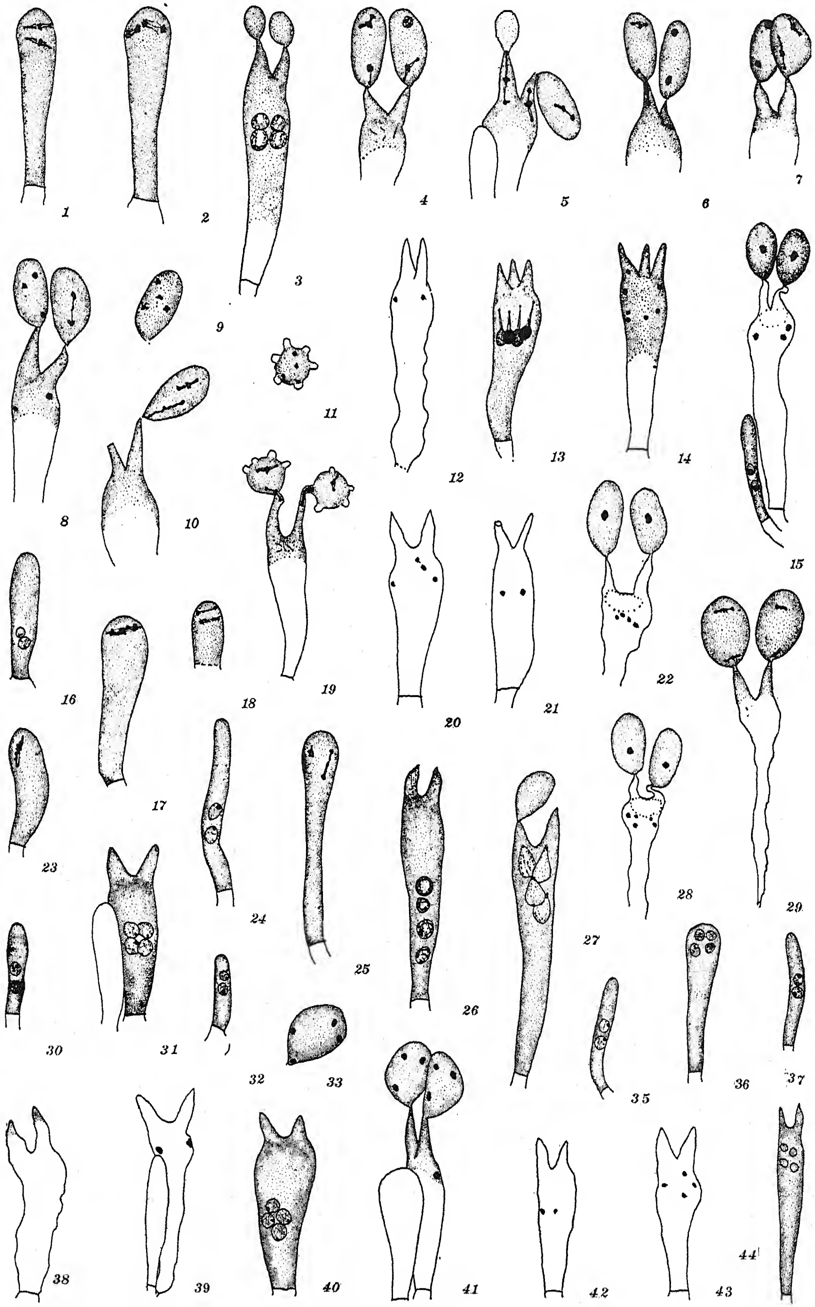
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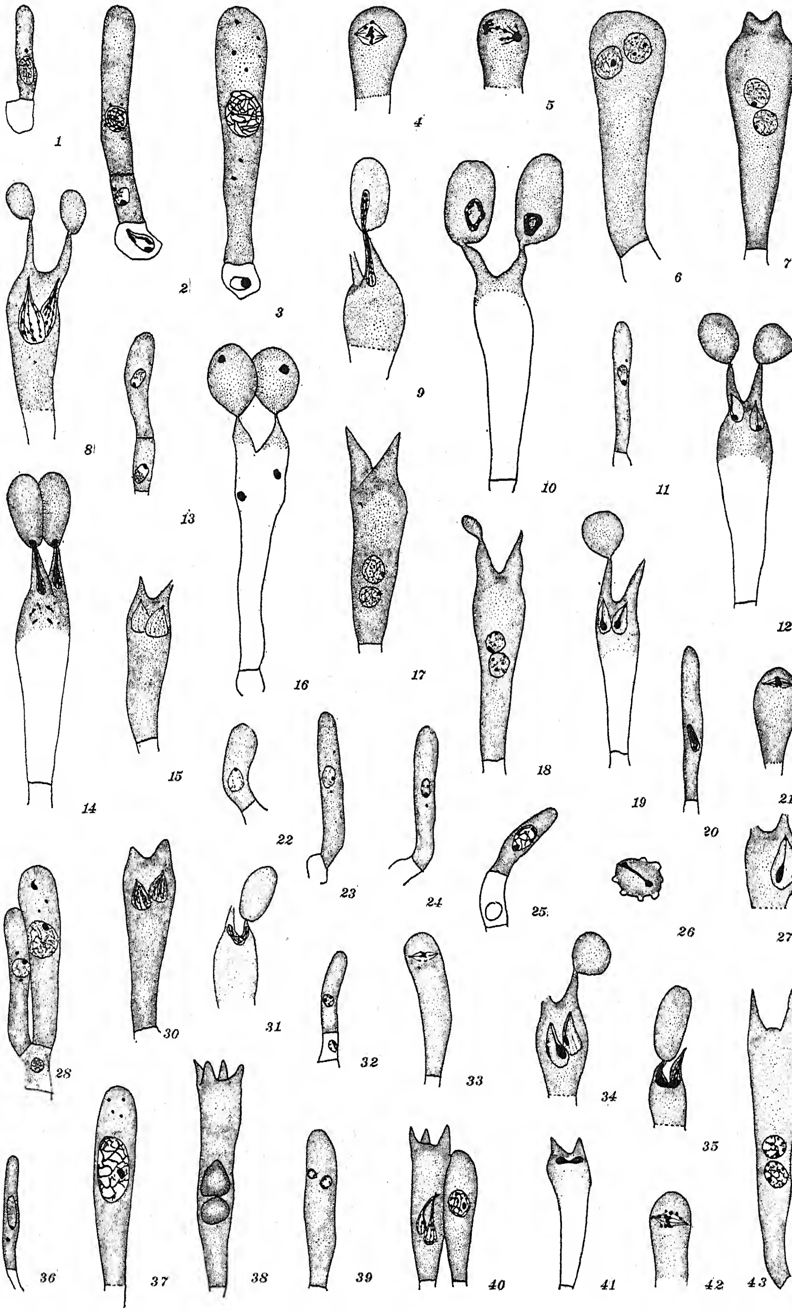
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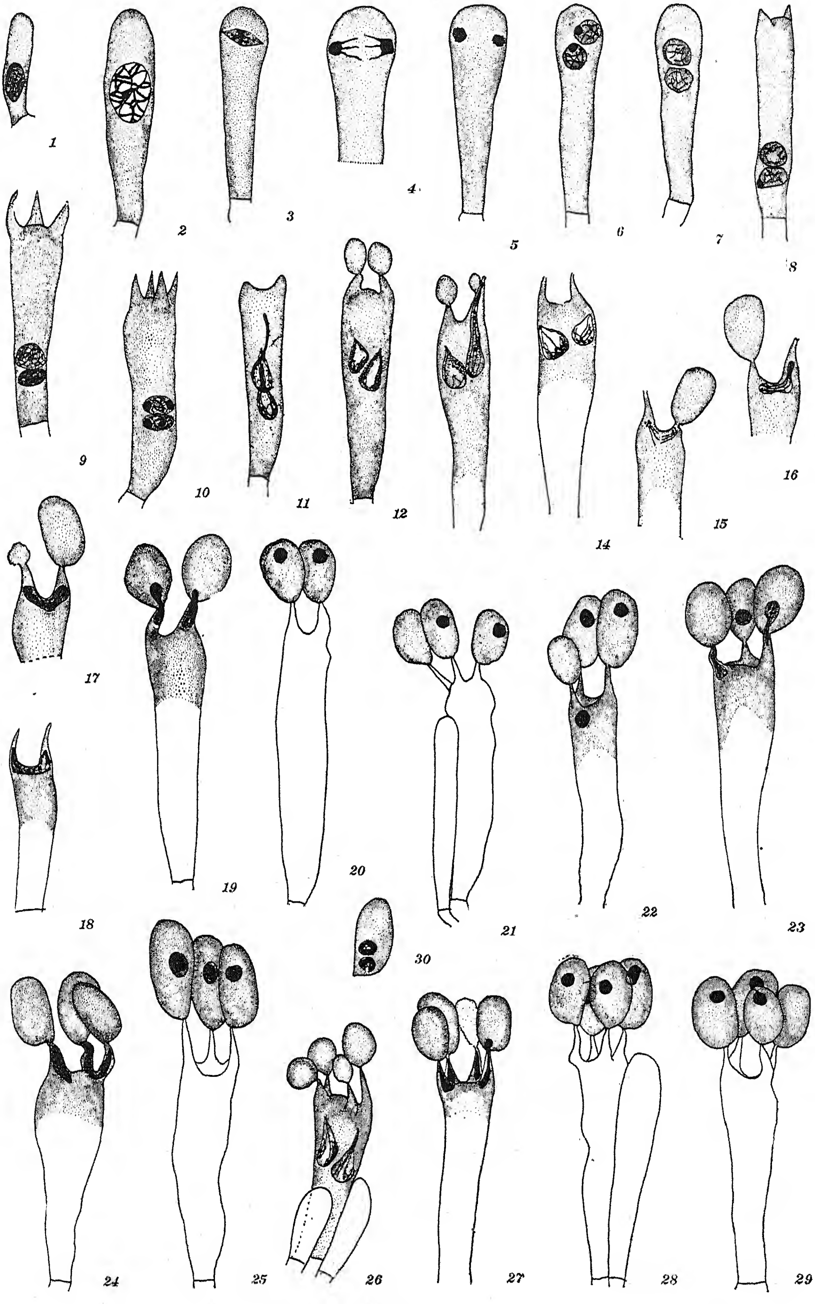

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THE GENUS MYXOMYCIDIUM [1]

David H. Linder

(with plate 39 and 1 text fig.)

The genus *Myxomycidium* was first described by Massee (4) from material sent to him from Tasmania by Rodway with the notation "Always pendulous and in consistency much more watery than any *Clavaria* we have." Doubt as to the position of the genus was expressed by Massee when he wrote "A remarkable genus without very evident affinities. The watery, gelatinous substance suggests the Tremellineae, but the narrowly clavate basidia, surmounted by four slender sterigmata, are opposed to this view. The general form of the sporophore is that of the Clavariae, where the gelatinous consistency is unknown." These remarks would indicate that he believed the organism to be more closely related to the Clavariaceae than to the Tremellineae, but Killermann (2) places it among the doubtful genera of the Tremellales.

In view of the apparent rarity and of the uncertainty concerning the taxonomic position of the genus, it seems worth while to record the observations made by the writer on material collected in British Guiana, South America, in 1923. The material unfortunately was preserved in 4 per cent formaldehyde solution and therefore was not in the best condition for cytological study. Nevertheless, by choosing those basidia which showed the nuclear structure and position most clearly, a fairly good idea of nuclear behavior could be obtained. The preparations were made by crushing under a cover glass the material mounted in lactophenol-cotton-blue and picric nigrosin, or in lactophenol plus equal parts of cotton blue and safranin Y. However, in spite of the care taken in working out nuclear behavior, it is evident that the results cannot be accepted as conclusive until supported by the study of properly killed and fixed material.

[1] Contribution no. 127 from the Laboratories of Cryptogamic Botany of Harvard University.

The fruiting bodies (PLATE 39, FIG. 1) of the South American material were growing gregariously on the side of a moist decaying log which still retained its bark. The younger fruiting bodies appeared to be little more than a short white stipe with a firm, white, gelatinous, clavate enlargement at the apex. With increasing age, the fruiting body enlarged apically and became more watery-white and considerably softer, until finally it became so watery at maturity that it was almost impossible to touch it without it flowing around one's fingers and leaving the stipe behind. Just before this final stage of liquefaction, the fruiting body was tinged a dilute ochraceous yellow, the color apparently imparted by the numerous spores.

Longitudinal sections of the fruiting bodies, made from specimens embedded in celloidin and stained,[2] disclose the fact that the fructifications which externally appear to the naked eye to be relatively simple structures, are in reality clearly differentiated into three distinct layers except near the apex where there is a transition zone that is not so clearly marked. Beginning at the center, there is a core consisting of irregularly coiled and contorted hyphae that run lengthwise. They are derived from the closely aggregated, nearly parallel hyphae that make up the short stipe. Towards the apex, the curled and twisted hyphae of the core straighten out and radiate to form the inverted rounded-conical tip, at the surface of which the basidia are formed. These radiating hyphae of the apex of the fruiting body merge with, and are a continuation of, the hymenial and subhymenial layers that are so clearly differentiated in the broader basal part of the fructification. In this broader region the hyphae although branched and flexuous or undulate, run parallel to one another and at right angles to the axis of the core to form the loose subhymenial tissue that is 100 to 150 μ thick. Most of the cells in this zone are binucleate but none of them show any evidence of clamp connections. The ends of the hyphae of the subhymenial layer become richly branched and give rise to the third or hymenial layer, 25 to 40 μ thick, which is composed of basidia rather closely aggregated in a palisade-like layer that covers the entire periphery of the swollen part of the fruiting body.

[2] The writer wishes gratefully to acknowledge the assistance of Dr. Roy Whelden, who embedded and stained this material.

The basidia are of two types: simple and "proliferative." The simples ones, 15–23 × 4.5–7 μ, are not truly narrowly clavate as the dimensions would indicate, since the measurements are taken from the length and contrasted with the broadest part. It would be more accurate to give the shape, in outline, as spatulate since the base of the basidium is relatively narrow, 2–3 μ in diameter, and apically is inflated into a subglobose to ellipsoid head. The "proliferative" type (PLATE 39, FIG. 20, 22) consists of two parts which correspond to the hypobasidium and epibasidium of Neuhoff (**5**). The hypobasidium is formed in the same manner as the true basidium, but instead of continuing to form sterigmata, proceeds to the formation of a second basidium-like structure that actually produces the sterigmata. Both the simple basidia and the epibasidia bear four sterigmata and thus they resemble the corresponding structures of the higher Autobasidiomycetes. The hymenium of the smaller fruiting bodies is composed of a single layer of basidia, but with increasing age and as the first formed basidium reaches maturity, a side branch is produced just below the basidium and this in turn gives rise to another basidium. The process is repeated until several basidia are formed at the end of a single branch of the subhymenial hyphae (PLATE 39, FIG. 24).

Nuclear behavior, so far as it can be made out from the material available, presents in the one instance a behavior similar to that found in the Tremellales, in the other, resembling that of the higher basidiomycetes. As the terminal cell begins to swell to form the basidium, the paired nuclei migrate to a central position and then as they come together, there is formed on each nucleolus a short comma-like tail that is directed obliquely towards the wall at the point of contact of the two nuclei (PLATE 39, FIG. 5), and then as the two nuclei are about to fuse, the comma-like tails are directed towards the opposite ends of the basidium (PLATE 39, FIG. 6). The fusion nucleus, it would seem, immediately enters the prophase (PLATE 39, FIGS. 7, 8) in which condition it migrates towards the apical end of the basidium which started to enlarge prior to the fusion of the nuclei and has by now reached its full dimensions. When the nucleus reaches the apex of the basidium, the chromatin material which has been in threads that were arranged around the periphery of the nucleus, breaks up or shortens into eight rather

distinct chromosomes (PLATE 39, FIG. 9) after which process the spindle is formed at right angles to the axis of the basidium (PLATE 39, FIG. 10) and division is finally completed. This appears to be the reduction division and can easily be distinguished from the later divisions, not only by the broader and more conspicuous spindles and by the more numerous chromosome masses, probably eight in the diploid phase, but also by the direction in which the process takes place.

Following the reduction division, the daughter nuclei migrate toward the base of the inflated terminal part of the basidium, and there undergo the second division. This time the process is completed, not in an horizontal direction, but at an oblique or nearly vertical direction, the direction depending in part on the angle from which the spindles are viewed. In the majority of instances they are oblique and parallel (PLATE 39, FIGS. 11–13), although in one or two instances (PLATE 39, FIGS. 14, 15) it would appear that, although dividing obliquely in reference to the axis of the basidium, the daughter nuclei divide at right angles in reference to each other. Whether this discrepancy in nuclear behavior is a result of improper fixing or whether the nuclei normally divide in an irregular fashion it is difficult to state since it is at this stage of development that the material proves rather unfavorable for cytological study. At any rate, it can be said that prior to division of the daughter nuclei or during the process, the basidium, if it is to be of the "proliferative" type, begins to send out the slender terminal elongation (PLATE 39, FIG. 11) that is later to enlarge and become the epibasidium. Apparently the epibasidium is not formed until after the completion of nuclear division since the resulting four nuclei may still be seen (PLATE 39, FIG. 15) in the hypobasidium, whence they do not migrate until formation of the sterigmata is initiated (PLATE 39, FIG. 18) and then they do not always migrate at one time, but may travel outward in pairs. The simple basidium offers little difficulty in interpretation since it appears that the nuceli merely migrate from the base of the inflated head to the apex (PLATE 39, FIG. 17), although it has been impossible to find any correlation between the position of the nuclei and sterigma formation. It is clear, however, that the nuclei in migrating from the basidium or epibasidium, as the case may be,

lose considerable volume since when they next appear in the spores they are noticeably smaller.

The subsequent history of the basidiospores remains obscure. There is, however, indication that the nucleus may divide within the spore (PLATE 39, FIG. 2) and if that is true, it is possible to postulate that the basidiospores proceed to the formation of secondary spores in a manner comparable to the repeated spore formation in the Tremellales. Further evidence for repeated spore formation is offered by the presence of large numbers of basidiospores which are entirely devoid of content. Unfortunately these spore remnants are so transparent that it is impossible to discern any evidence of sterigmata.

In addition to the basidiospores, there are present on the fruiting body larger spores of essentially the same shape, but characterized by the presence of two or more nuclei. Such spores, although abundant in two or three of the fruiting bodies, were never found attached to any definite structure, and perhaps they may prove to be of foreign origin. In only one instance was any comparable structure found without question to be attached to the hyphae of the fruiting body (PLATE 39, FIG. 4), but that was so immature that the writer hesitates to conclude that it is the same as the large several-nucleate spore, especially since obviously foreign spores are also present.

The relationship of the South American species of *Myxomycidium* is clearly not with the Tremellales in the narrower sense of the term since septa are lacking in the basidia and also because instead of both divisions of the fusion nucelus taking place in a more or less horizontal plane, as in the majority of the species of the Tremellaceae, only the first division is horizontal. There are, however, many instances in which the septa of the tremellaceous fungi are laid down irregularly instead of vertically and this would indicate that not all divisions of the nuclei are in an horizontal plane. Lack of septations in the hypobasidium, then, would prevent this species from being placed in the Tremellaceae. On the other hand, the jelly-like consistency of the fruiting body would seemingly exclude the species from the Clavariaceae even though there is present a well differentiated hymenial layer. For the sake of brevity and clarity, the differences between *Myxomycidium*

and members of the chiastobasidial Clavariaceae are tabulated below. Obviously the stichobasidial *Clavarias* need not be considered since the first division of the nucleus is parallel to the long axis of the basidium.

Myxomycidium (South American)	Clavariaceae (chiastobasidial)
Fructification soft, gelatinous.	Fructification brittle to firm, fleshy to fibrous.
All hyphae in a gelatinous matrix.	No evidence of gelatinous matrix.
Subhymenium of nearly parallel hyphae.	Subhymenium of intricately intertwined hyphae.
Basidia conspicuously inflated at the apex.	Basidia mostly narrowly clavate.
Basidium production indeterminate, acropetalous.	Basidium production determinate.
Epibasidia often produced.	No epibasidia produced.
First division of fusion nucleus horizontal, second oblique, nearly vertical.	First and second divisions horizontal.

If weight is placed on the indeterminate acropetalous production of basidia, on nuclear behavior, and on the frequent production of epibasidia, then it becomes impossible to place *Myxomycidium* in the Clavariaceae for there is little agreement between the two. Most of the characters of the genus are more primitive than would be expected in the Clavariaceae.

The Vuilleminaceae, with *Vuilleminia comedans* (Nees) Maire (3) as the type, offers a possible solution. Although the fructifications of *Vuilleminia* are resupinate, they are also gelatinous. Furthermore, each hypobasidium produces a single clavate epibasidium in which the meiotic division takes place at right angles to the long axis of the basidium. The subhymenium of *Vuilleminia* and *Myxomycidium* agree fairly well, and since both genera are gelatinous in texture, the latter genus could be considered a stipitate form derived from a resupinate one, or possibly from an as yet undiscovered pustulate form. The basidia also are strikingly similar, but they differ in certain respects. In *Vuilleminia* the fusion nucleus migrates from the hypobasidium into the epibasidium and there undergoes division, whereas in *Myxomycidium* the fusion nucleus usually divides in the hypobasidium and passes after the second division into the epibasidium. Occasionally, however, instances may be observed (PLATE 39, FIG. 23) in which the

fusion nucleus migrates into the epibasidium and there divides. Still another difference is the presence of the simple type of basidium, but this difference is more apparent than real since intergradations between the two types are readily found (PLATE 39, FIG. 22, 23, 18, 12, 19). Even though there is a degree of variation in the morphology of the basidium, the fact that the vuilleminaceous type is produced is of considerable significance and points to the relationship of *Myxomycidium* to *Vuilleminia* rather than to the Clavariaceae, in which family the basidia appear to be morphologically rather constant.

The above characters point to a relationship with the Vuilleminaceae, but against this is the evidence presented by the behavior of the basidiospores, and by the presence of the larger conidium-like spores. According to Maire (3), the basidiospores of *Vuilleminia* become septate at maturity as a result of nuclear division and the laying down of a cross-wall, whereas those of *Myxomycidium* apparently remain one-celled and may possibly proliferate with the production of secondary spores. Also, although conidia are reported frequently in the Tremellaceae and occasionally in the Clavariaceae as shown by Coker's figure (1) of *Clavaria botrytis,* none have been reported in *Vuilleminia.* Since Maire was primarily interested in the cytological study of the basidium, it is possible that he overlooked the conidial stage, but even if conidia prove to be absent in that genus, it is not a point on which too great stress should be placed since in the genera of the Tremellaceae and Clavariaceae in which conidia have been reported, species occur which lack such reproductive bodies. There remains then only basidiospore behavior as an ojection to placing *Myxomycidium* in the Vuilleminaceae, but since most of the other characters indicate such a relationship to be possible, it would seem logical to include the genus in that family.

The identity of the South American and Tasmanian specimens, because of the differences in the dimensions of the basidia and spores of the two specimens, was somewhat doubtful, and the doubt was increased by the finding in Dr. Thaxter's collection of an additional specimen from Tennessee, which differed from the South American material by the presence of clamp connections at or below the basidia. Such conspicuous differences between the

North and South American material showed the desirability of studying type material, and this, through the kindness of Mr. A. D. Cotton and Miss E. M. Wakefield of the Kew Herbarium, the writer was able to do. A comparison of the three specimens definitely proved the fact that instead of dealing with one variable species, there were three to be considered. The differences between them may be indicated briefly by the following key:

1. Basidia clavate, not conspicuously narrowed below, (13.5)–25–28 × 5–7 μ; sterigmata 2 or 4, 2.5–4 μ long; spores (5)–6–7 × 4.5–5 μ ... *M. pendulum.*
1. Basidia conspicuously narrowed below; sterigmata 4; spores 5–6.5 × 3–3.5 μ ... 2.
2. Clamp connections absent; hypobasidia often present; sterigmata short, 2.5–4 μ ... *M. guianense.*
2. Clamp connection present; hypobasidia seldom present; sterigmata long, 5.5–9 μ ... *M. nodosum.*

MYXOMYCIDIUM PENDULUM Massee, Kew Bull. Mis. Inf. **1899**: 180. 1901.

Receptacula pendula, aquoso-gelatinosa, stipitata, lanceolata, apice acuta, hyalina vel basi ochraceo tincta, 1–1.5 cm. longa. Basidia clavata, (13.5)–25–28 × 5–7 μ, leniter ad bases constricta ubi 3.5–4 μ diametro; sterigmata 2 vel 4, 2.5–4 μ longa. Sporae subsphaericae vel ovoidea, inaequilaterales, apiculatae, hyalinae, glabrae, (5)–6–7 × 4.5–5 μ.

Fruiting body pendulous, watery gelatinous, stipitate, lanceolate with an acute apex, hyaline or dilute ochraceous at the base, 1–1.5 cm. long. Basidia clavate, slightly constricted towards the base, (13.5)–25–28 × 5–7 μ, tapering basally to 3.5–4 μ in diameter; sterigmata 4, less frequently 2, stout, tapering, 2.5–4 μ long. Spores subspherical to ovoid, inequilateral and usually obliquely apiculate, hyaline, smooth, (5)–6–7 × 4.5–5 μ.

Tasmania: on rotten wood, *Rodway, 605,* TYPE.

This species differs from the two following species in the proportions and dimensions of the basidia and spores. The basidia are more nearly sessile on the subtending hyphae, and although crowded in the hymenium, they are produced indeterminately and acropetalously as in the other species. Neither clamp connections nor hypobasidia were observed.

The sizes of the basidiospores as given by Massee (8–9 × 6 μ) are somewhat larger than those obtained by the writer from meas-

urements of spores from the type material. The differences may result from the fact that Massee had fresh or nearly fresh material at his disposal, whereas the present studies were made from material that had been dried for over thirty years and then mounted in lactophenol. Should Massee's measurements prove to be correct for fresh material, then the difference between this and the two following species, preserved (after having been killed in 4 per cent formalin solution) since they were collected in alcohol and glycerine, is more pronounced than it would appear from the above description.

Myxomycidium guianense sp. nov.

Receptacula pendula, aquoso-gelatinosa, breve stipitata, lanceolata, apice acuta, primum hyalina deinde ochraceo tincta, 1–3 cm. longa. Basidia 15–23 $\times$ 4.5–5.5–(7) μ, clavata, manifesto ad bases constricta ubi 1.5–2 μ diametro, saepissime ex hypobasidiis orientia; sterigmata 4 raro 3, brevia, fastigata, 2.5–4 μ longa. Sporae ovoideae vel ellipsoideae, subinaequilaterales obliquiterque apiculatae, hyalinae, glabae, 5.5–6.5 $\times$ 2.5–3.5 μ.

Fruiting bodies pendulous, watery-gelatinous, short-stipitate, lanceolate, the apices acute, at first hyaline or milky-white becoming ochraceous tinged, 1–3 cm. long. Basidia 15–23 $\times$ 4.5–5.5–(7) μ, clavate, conspicuous, constricted towards the base where they measure 1.5–2 μ in diameter, frequently arising from hypobasidia; the sterigmata 4, short and tapering, 2.5–4 μ long. The basidiospores ovoid or ellipsoid, subinequilateral and obliquely apiculate, hyaline, smooth, 5.5–6.5 $\times$ 2.5–3.5 μ.

British Guiana; Bartica, on decaying fallen tree trunk, December, 1923, *Linder, 456* and *624,* TYPE.

Myxomycidium nodosum sp. nov.

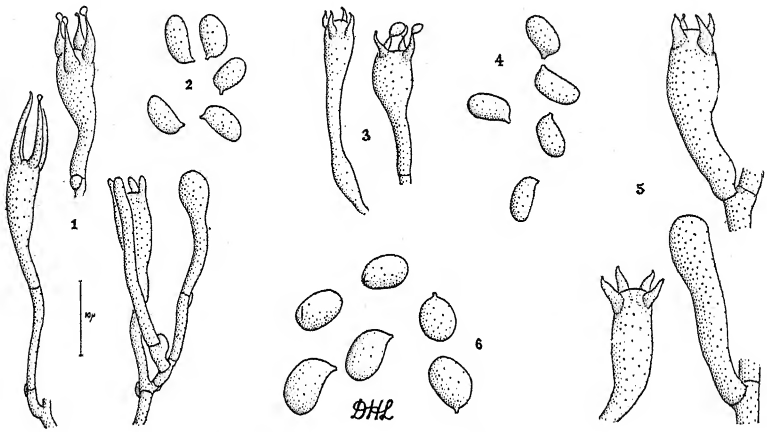
Receptacula pendula, aquoso-gelatinosa, breve stipitata, lanceolata, apice acuta, primum hyaline (?) deinde viridi-ochraceo tincta, 1–3 cm. longa. Basidia 16.5–25.5 $\times$ 4–5 μ, clavata, manifesto ad bases constricta ubi 1.5–2 μ diametro, raro ex hypobasidiis orientia; sterigmata 4, elongata fastigataque, 5.5–9 μ longa. Sporae ovoideae vel ellipsoideae, subinaequilaterales obliquiterque apiculatae, hyalinae, glabrae, 5–6 $\times$ 3–3.5 μ.

Fruiting bodies pendulous, watery gelatinous, short-stipitate, lanceolate, the apices acute, at first hyaline or milky white (?) becoming tinged greenish-ochraceous, 1–3 cm. long. Basidia 16.5–25.5 $\times$ 4–5 μ, clavate, conspicuously tapering towards the base

where they measure 1.5–2 μ in diameter, rarely arising from hypobasidia; the sterigmata 4, 5.5–9 μ long and tapering towards the apex. The basidiospores ovoid or ellipsoid, subinequilateral and obliquely apiculate, hyaline, smooth, 5–6 × 3–3.5 μ.

Tennessee: Burbank, on decaying wood, August 1896, *R. Thaxter*, TYPE.

M. nodosum, in gross appearance, very closely resembles *M. guianense* although because of the greenish color, as shown by Dr. Thaxter's water color drawings, the two species might possibly



FIGS. 1–2. *Myxomycidium nodosum* to show characteristic basidia with elongate sterigmata, the nodose-septate hyphae, and basidiospores; 3–4, *M. guianense*. Basidia and basidiospores. In figure 3, the left hand basidium is shown arising from a hypobasidium; 4–5, *M. pendulum* to show the clavate and but slightly narrowed, almost sessile basidia and the larger ovoid or subspherical basidiospores. The drawings were made from material mounted in lactophenol and are reproduced at a magnification of approximately × 1100.

be distinguished without resort to the microscope. Both of these species are alike in the structure of the context: the hyphae are rather slender and the context is differentiated into the three zones that are mentioned in an earlier paragraph. The constricted basidia and the shape of the spores would indicate that these two species are more closely related to each other than to *M. pendulum*, which produces slightly constricted basidia, and coarser and more densely arranged hyphae in the context. The presence of hypo-

basidia and the lack of clamp connections clearly differentiate *M. guianense* and *M. nodosum*, but whether the comparative lengths of the sterigmata may be relied on as of taxonomic value may be open to discussion. Certainly in the material examined, the sterigmata of *M. nodosum* were predominantly long, while those of *M. guianense* were predominantly short, but in a few cases the sterigmata of apparently abnormal basidia of *M. guianense* were as long as those of *M. nodosum*, although bluntly rounded at the tips.

The types of the two new species are deposited in the Farlow Herbarium of Harvard University, as is a microscopical preparation from the type of *M. pendulum* which is at Kew. Cotype specimens are also at The New York Botanical Garden.

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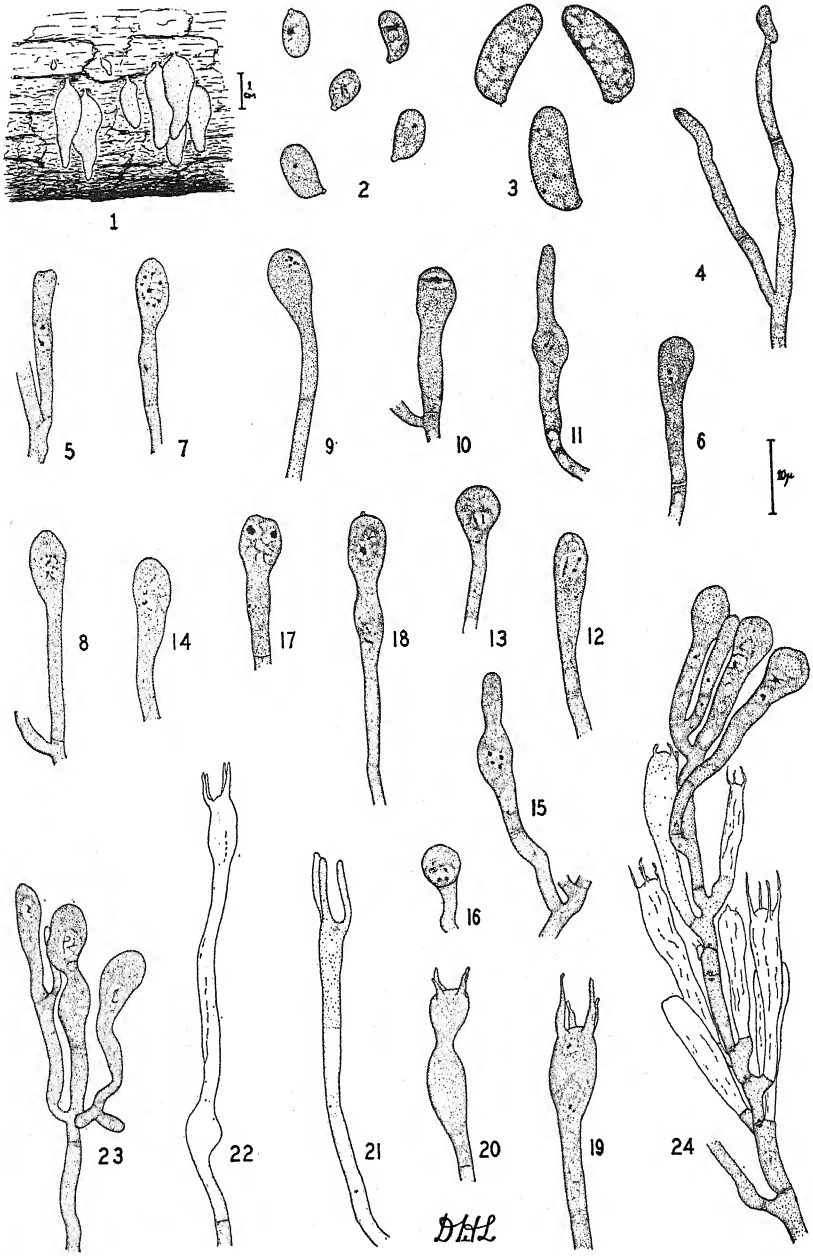
EXPLANATION OF PLATE 39

All drawings are of *Myxomycidium guianense* and with the exception of figure 1, were made with the aid of a camera lucida from material mounted in lactophenol, and as reproduced are shown at a magnification of approximately $\times$ 1125.

Fig. 1, Fruiting bodies at various stages of development to show the gregarious manner of growth. Drawing made in the field from living material. $\times \frac{1}{2}$; 2, Basidiospores showing the single nucleus and the rather prominent apiculus. The nucleus of the spore in the center of the group appears to be in the process of division; 3, Large multinucleate conidia that apparently belong in the life cycle of *M. guianense*. The upper right hand conidium shows a slight scar behind the apiculus,—the point of attachment of the conidium; 4, A branch from a basidium-bearing hypha on which is formed a structure that appears to be an immature conidium; 5, A young basidium showing the paired nuclei in contact, and the nucleoli with their comma-like tails directed obliquely towards the wall of the basidium; 6, A basidium in which the nuclei, but not the nucleoli, have fused. The apical portion of the basidium has already begun to enlarge; 7–8, Basidia showing the nuclei in the prophase condition. Note the peripheral arrangement of the chromatin bodies; 9, Basidium showing the fusion nucleus in the late prophase condition, at which stage eight chromosomes are evident; 10, Showing the transversely arranged spindle of the meiotic division; 11, Hypobasidium



The Genus Myxomycidium

showing the obliquely dividing daughter nuclei and the formation of the elongate process that is to become the epibasidium; 12–14, The division of the daughter nuclei in the simple type of basidium. In figure 14 the nuclei are dividing at right angles to each other; 15, Four daughter nuclei in the hypobasidium which has not yet completed the formation of the epibasidium; 16–18, The simple type of basidium with four nuclei. In figure 16 the basidium is curved upwards and shows the arrangement of the nuclei as seen from above. In figures 17 and 18 the sterigmata are just beginning to form; 19, A simple basidium with four sterigmata. One nucleus can be seen as it is about to enter the sterigma above it; 20, A basidium which is only slightly constricted into an epi- and hypobasidium; 21, An apparently abortive basidium with only three stout sterigmata borne on the but slightly inflated apical region; 22, A discharged epibasidium borne on an elongate projection from the hypobasidium; 23–24, To show the characteristic method of branching and the indeterminate production of basidia by a single branch of the subhymenial hypha.

PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI—XXI. THE GENUS CALYCINA[1]

FRED J. SEAVER

(WITH PLATE 40)

The above named genus was established by S. F. Gray with *Peziza firma* of Persoon as the first named species and, therefore, regarded as the type, although the genus included a number of other species not now regarded as congeneric. The genus was pre-Friesian and has apparently never been used as a valid genus since. The writer, therefore, proposes to make this genus, as typified by *Peziza firma,* post-Friesian and to include in it certain other species which in his opinion are congeneric with the proposed type.

Specimens which seem referable to *Peziza firma* Pers. have been frequently collected in North America. Probably the most commonly collected species here regarded as congeneric with the type is the so-called *Geopyxis nebulosa.* Although very often sent in for determination it is difficult to designate since it is not a *Geopyxis,* neither is *nebulosa* the correct specific name, the species having been previously described by Peck under the name *Helotium macrosporum,* a very fitting name since the large size of the spores is one of the most striking characteristics of the species.

The proposed genus is most closely related to *Ciboria* of Fuckel which again contains species which are not now regarded as congeneric. One of the characteristics of the species of the genus *Calycina* is the possession of spores which become tardily septate, the number of septa varying. Also the spores often produce small knobs or buds at one or both ends although these are not always present. Below is presented the writer's conception of the genus and the included species.

[1] This paper is preliminary to a monograph of North American Cup-fungi (inoperculates), a companion volume to North American Cup-fungi (operculates), which was published by the author and issued in December, 1928.

CALYCINA S. F. Gray, Nat. Arrang. Brit. Pl. **1**: 669. 1821.

Ciboria, Fuckel Symb. Myc. 311. 1869. (in part only).

Apothecia medium large, up to 1 cm. in diameter stipitate, or subsessile, the length of the stem varying with the conditions, light-colored or dull, externally smooth or with very poorly developed hair-like structures; asci cylindric or clavate, usually 8-spored; spores ellipsoid to fusoid, hyaline, for a long time simple later often becoming septate with one to several septa.

Spores comparatively small, not exceeding 20 μ long.
  Substance of apothecia not golden-yellow. ....1. *C. firma.*
  Substance of apothecia when crushed golden-yellow. ......2. *C. bolaris.*
Spores comparatively large, 30–35 μ long. ....3. *C. macrospora.*

1. CALYCINA FIRMA (Pers.) S. F. Gray, Nat. Arrang. Brit. Pl. **1**: 669. 1821.

? *Peziza ochroleuca* Bolton, Fungi Halifax **3**: 105. 1789.
Peziza firma Pers. Syn. Fung. 658. 1801.
Ciboria firma Fuckel, Symb. Myc. 312. 1869.
Helotium firmum Karst. Not. Soc. Fauna Fl. Fenn. **11**: 233. 1871.
Rutstroemia firma Karst. Not. Soc. Fauna Fl. Fenn. **13**: 233. 1873.
Phialea firma Gill. Champ. Fr. Discom. 101. 1882.
Hymenoscypha firma Phill. Brit. Discom. 123. 1893.
Ciboria ochroleuca Massee, Brit. Fungus Fl. **4**: 274. 1895.

Apothecia gregarious, infundibuliform, becoming expanded and often nearly discoid, stipitate, brownish reaching a diameter of 1 cm. though often smaller; hymenium brown, darker than the outside of the apothecium; stem variable in length but up to 12 mm. and .5 mm. in diameter but gradually expanding above; asci cylindric or subcylindric reaching a length of 130 μ and a diameter of 9–12 μ, 8-spored; spores ellipsoid or fusoid obliquely 1-seriate with the ends overlapping, $4–6 \times 15–20\ \mu$, at first simple, occasionally becoming 1–3-septate; paraphyses filiform, enlarged, above, reaching a diameter of 2 μ.

On woods of various kinds.

TYPE LOCALITY: Europe.

DISTRIBUTION: New York and New Hampshire to Colorado; also in Europe.

ILLUSTRATIONS: ? Bolton Hist. Fung. **3**: *pl. 105;* Boud. Ic. Myc. *pl. 483;* Gill. Champ. Fr. Discom. *pl. 74, f. 2;* E. & P. Nat. Pfl. 195, *f. 155. O, P;* Sow. Engl. Fungi *pl. 115.*

2. **Calycina bolaris** (Batsch) Seaver, comb. nov.

Peziza bolaris Batsch, Elench Fung. Cont. **1**: 221. 1786?
Ciboria bolaris Fuckel, Symb. Myc. 311. 1869.
Phialea bolaris Boud. Bull. Soc. Myc. Fr. **1**: 116. 1885.
Hymenoscypha bolaris Phill. Brit. Discom. 124. 1893.

Apothecia gregarious, stipitate or subsessile, expanding and becoming discoid or slightly convex, externally yellowish, reaching a diameter of 5–6 mm. with a few club-shaped hair-like structures about the margin; hymenium slightly concave, plane or a little convex, yellowish brown, a little darker than the outside of the apothecium; (substance of the apothecia when crushed, golden yellow); stem short, usually a little less than the diameter of the apothecium, about .5 mm. in diameter, expanding rather abruptly into the apothecium; asci cylindric or clavate, 8-spored reaching a length of 200 μ and a diameter of 12–14 μ; spores ellipsoid, usually slightly curved, 7–9 $\times$ 18–20 μ, for some time simple, finally 1-septate and often with apiculi at one or both ends, later 3-septate; paraphyses filiform, enlarged above, the contents yellow.

On twigs of various kinds.

TYPE LOCALITY: Europe.

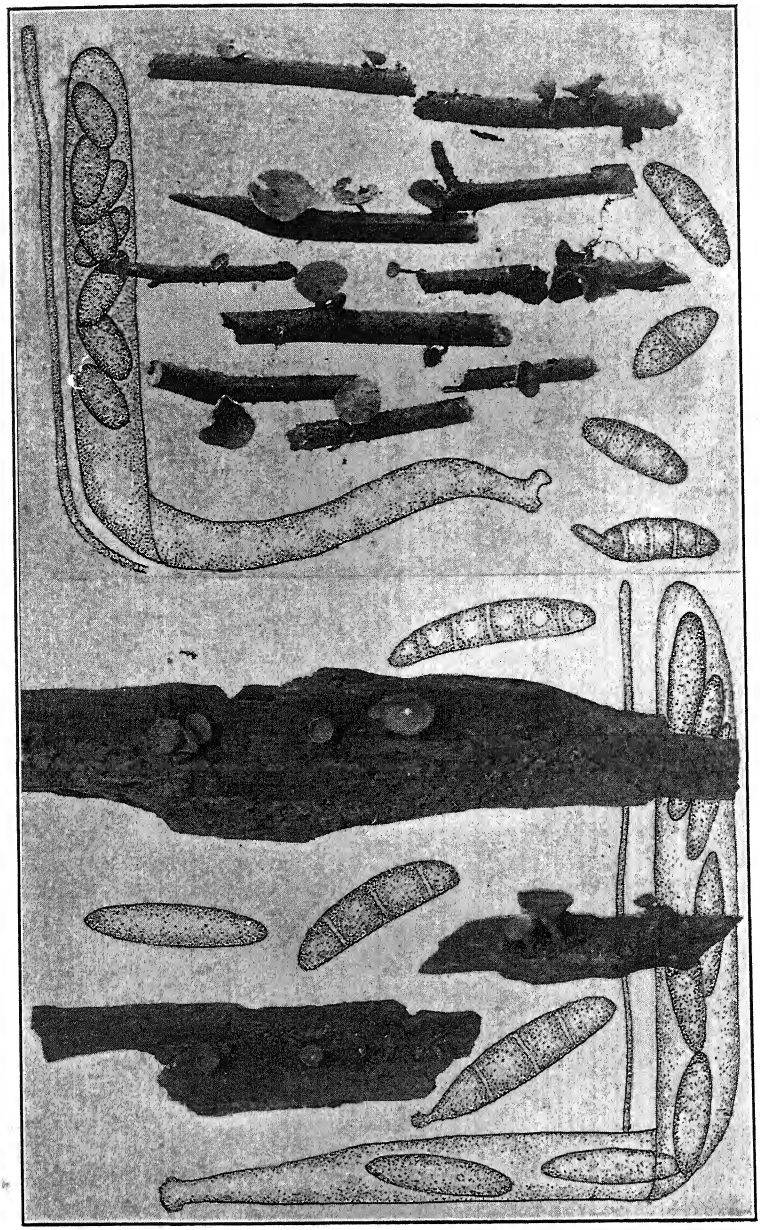
DISTRIBUTION: New York; also in Europe.

ILLUSTRATIONS: Ic. Myc. *pl. 482.*

This species is close to *Calycina firma* but seems to differ in that it is less robust and lighter colored, yellowish instead of brownish. The only American specimen seen is one collected by H. H. Whetzel, No. *10784.*

3. **Calycina macrospora** (Peck) Seaver, comb. nov.

Helotium macrosporum Peck, Ann. Rep. N. Y. State Mus. **26**: 82. 1874.
Peziza nebulosa Cooke, Mycographia 163. 1877.
Geopyxis nebulosa Sacc. Syll. Fung. **8**: 70. 1889.
Ciboria Dallasiana Ellis & Ev. Jour. Myc. **9**: 165. 1903.
Tarzetta cinerascens Rehm, Ascom. *1853;* Ann. Myc. **2**: 352. 1904.
Ciboria fuscocinerea Rehm, Ann. Myc. **7**: 525. 1909.



Calycina bolaris
Calycina macrospora

Apothecia gregarious, stipitate or occasionally subsessile, at first closed and subglobose, gradually expanding and becoming nearly discoid, attenuated at the base, reaching a diameter of 1 cm., cinereous to yellowish-brown; hymenium concave or nearly plane, similar in color to the outside of the apothecium; stem reaching a length of 1 cm. and a diameter of about 1 mm.; asci cylindric or clavate, 8-spored, reaching a length of 150 μ and a diameter of 10–12 μ; spores narrow ellipsoid or fusoid, straight or slightly curved, hyaline granular, for a long time simple, finally becoming 1- to 5-septate and often with an apiculus at one or both ends and an oil-drop between each two septa, reaching a length of 30–35 μ and a diameter of 6–7 μ; paraphyses filiform, slightly enlarged above.

On rotten wood.

TYPE LOCALITY: South Carolina.

DISTRIBUTION: New York to Virginia, North Dakota and Colorado.

ILLUSTRATIONS: Cooke Mycographia *pl. 73, f. 281;* Seaver, Iowa Discom. *pl. 20, f. 2.*

EXSICCATI: N. Am. Fungi *477;* Rehm, Ascom. *1853;* Seaver, North Dakota Fungi *6.*

THE NEW YORK BOTANICAL GARDEN.

EXPLANATION OF PLATE 40

Upper figure. *Calycina bolaris.* Photographs of several apothecia about natural size. At the left, drawing of ascus with spores and paraphysis. At the right, several spores. Photograph furnished by H. H. Whetzel.

Lower figure. *Calycina macrospora.* Photograph of several apothecia about natural size, with drawings of ascus with spores and paraphysis. Photograph furnished by the Dominion National Museum, Canada.

NOTES ON BOLETES. III.

Walter H. Snell

(with plate 41)

Boletus mutabilis = *Boletus pulverulentus*

Boletus mutabilis was described by Morgan from Ohio in 1884. So far as I know, it has not been found by anyone since, but there is no question at all but that there are many species of boletes in other parts of this country than Peck's New York and Frost's New England that have never been reported, because of lack of any wide interest in these plants.

Murrill in listing this as a doubtful species,[1] said that he had not seen the types, but that Peck's New York plants are either *B. sordidus* or *B. felleus*. I do not remember whether Peck's plants were collected by him or were sent by Morgan, but in either case it seems strange that either of them would identify a true *B. sordidus* or *B. felleus* as *B. mutabilis*. Any bolete sporophore that could be called *B. mutabilis* is far from either *B. sordidus* (which I have said previously [2] may be *B. porphyrosporus,* but which perhaps is not) or *B. felleus*. Morgan's plant is characterized especially by the bright yellow stipe, tubes and flesh, with the tubes and flesh *promptly changing to deep blue*. The dark brown *B. sordidus* with light colored flesh apparently not changing to blue and the distinctive *B. felleus* with white, unchanging flesh, reticulate stipe and flesh-colored tubes are about as far removed from the plant described by Morgan as could possibly be imagined.

Inasmuch as the really distinctive feature of *B. mutabilis* as described by Morgan is the sudden changing of the flesh and tubes to blue when cut or bruised, it was most natural for one to be reminded of the extremely sensitive European *B. pulverulentus*. When descriptions of these two plants are compared, it is found that they read almost word for word, one for the other. As far

[1] Boletaceae of North America. Mycologia 1: 16. 1909.

[2] Notes on Boletes. I. Mycologia 24: 336. 1932.

as one can rely upon descriptions alone, it, therefore, appears that *B. mutabilis* Morgan is nothing else than *B. pulverulentus* Opat., a plant never reported as occurring in this country.

BOLETUS ROSEOTINCTUS

This species was considered by Murrill [3] to be the same as *B. Peckii* Frost, perhaps because both have a rosy-tinted pileus. I have collected what I believe to be *B. roseotinctus,* which agrees with Peck's description except for size and the stratifications of the flesh of the pileus. Examination of the specimens at Albany showed that there were specimens there larger than the dimensions given by Peck and that while some of the sporophores showed a stratification of the flesh, others did not (in the dried condition, of course).

B. roseotinctus could hardly be the same as *B. Peckii.* This latter species has a sporophore characterized by a reticulate stipe and whitish flesh changing to blue, while *B. roseotinctus* has an even, furfuraceous stipe and unchanging yellow flesh. This latter species resembles *B. subglabripes* Peck more than it does anything else (except for the pinkish color), but the spores of *B. roseotinctus* are more elliptical than subfusiform and those of *B. subglabripes* are subfusiform and not at all elliptical.

I consider *B. roseotinctus* a valid species.

THE VERSIPELLIS-SCABER COMPLEX

The tribe Versipelles of Fries contains the species with tubes white or whitish and minute, and the tube layer becoming nearly free. The American species which have been placed in this tribe are the following: *chromapes* Frost, *versipellis* Fries, *scaber* Fries, *niveus* Fries, *albellus* Peck, and *subpunctipes* Peck.

The striking and beautiful *B. chromapes* with pinkish pileus and yellow-footed stipe is readily identified. On the other hand, the remaining species of the tribe have offered considerable difficulty.

Boletus subpunctipes was described by Peck as differing from *B. versipellis* and *B. scaber* by:—the uneven, dry surface; tubes *adnate* and changing to reddish-brown where wounded; the stipe

[3] Loc. cit., p. 151.

slightly reticulate at the apex and *very minutely dotted;* spores rusty-brown or cinnamon-brown in mass instead of snuff-brown, and smaller than those of the two species last named. These differences seem to be of a minor nature, and one wonders if perhaps Peck did not have an aberrant or a faded form of *B. chromapes,* but nothing definite can be said until further studies can be made.

Boletus albellus is distinguished from *B. niveus,* the other white species, and from all the other species of this tribe, by the glabrous or nearly glabrous stipe.

The remaining species have been the subject of much controversy among mycologists. One extreme of the solutions offered is that of Murrill's, who considered *B. scaber* and *B. versipellis* the same as *B. viscidus* of Fries. It seems certain that *B. versipellis* and *B. scaber* are not the same, and they are nowhere near *B. viscidus,* which includes the *B. elbensis-B. laricinus* complex (tribe Viscipelles). As the other extreme, Fries distinguished, along with some other European forms, *B. versipellis, B. scaber, B. niveus* and *B. rugosus* (*B. leucophaeus* Pers.) and made *B. aurantiacus* (*B. rufus* of many workers) a variety of *B. scaber.* Gilbert and other French workers, including Peltereau, are very insistent that these latter species are different, but they cannot, however, follow Fries entirely. They believe that *B. aurantiacus* is not a variety of *B. scaber,* but a distinct species and they cannot be sure about *B. versipellis.* Gilbert [4] says that apparently Fries himself had no precise idea what *B. versipellis* was and that he changed his concepts of it in successive treatises. Therefore, because of this uncertainty and confusion, he would call it *B. floccopodus* (in his arrangement, *Krombholzia floccopoda*). He chose this specific name because it is something like *floccopus* under which name Rostkovius described the species, but which will not hold for *B. versipellis,* because *B. floccopus* is *Strobilomyces strobilaceus.*

In the light of the foregoing confusion, I have made some study of these forms macroscopically and microscopically and I am inclined pretty much to agree with Gilbert. I believe that the following species can readily be distinguished in the northeastern

[4] Personal correspondence and "Les Bolets," p. 183. Paris. 1931.

states:—*aurantiacus, versipellis* (or if one prefers, *floccopodus*), *scaber, leucophaeus* and *niveus.* I believe they can be distinguished as follows:—

Boletus aurantiacus—pileus usually more orange than *B. versipellis,* although often not, and not so reddish as described and portrayed by Europeans; surface dry, *tomentose;* margin always appendiculate with the projecting pellicle (not a veil); flesh white, changing to roseate and then to violaceous-rosy; stipe with asperities at first white, *then reddish, finally brownish;* spores *elongate-elliptical* or *narrowly subfusiform,* pale greenish under the microscope, and 13–15 × 3–3.5 μ; cystidia common, ventricose, globose-papillate or fusiform; probably the only one occurring under conifers as well as under poplars and birches; rare.

Boletus versipellis (or *floccopodus*)—pileus usually more orange-*yellow;* surface dull, unpolished, *glabrous* to more or less fibrillose-granulose under a lens; margin, color-changes of flesh, and cystidia as in *B. aurantiacus;* spores *elliptic*-fusiform, pale greenish under the microscope, 11–16 × 3.5–4 μ; occasional under birches.

Boletus scaber—pileus grayish or isabelline to brownish; surface glabrous, usually viscid; margin *usually not appendiculate,* but occasionally so; flesh white becoming more or less grayish or dingy violaceous; stipe with asperities at first white, then grayish and finally blackish; spores mostly *broadly elliptical* to *fusiform-elliptical* (not so fusiform as those of *B. versipellis*), *olivaceous-brown under the microscope,* 13–21 × 4–7 μ, mostly 16–18 × 4–6 μ, but larger or smaller, varying with the specimen, some as long and some as broad as those of *B. leucophaeus,* but none both as long and as broad; cystidia rare or lacking, ventricose-rostrate to clavate; very common under birches.

Boletus leucophaeus—pileus *dark-brown* to *blackish,* disc usually black; surface dry, strongly *tomentose;* white flesh becoming grayish or blackish; margin not appendiculate as far as observed; stipe somewhat darker than that of *B. scaber,* more rugose, with asperities nearly black; spores olivaceous brown under microscope, elliptical or subfusiform, *large,* 17–21 × 6–7 μ; cystidia not found; rare, under birches in mixed hardwoods and conifers (as far as observed).

Boletus niveus—pileus white or whitish; flesh *very firm* becoming more or less blackish; stipe scurfy or appressed scaly, but not so pronouncedly as the stipes of other species; spores olivaceous brown under the microscope, fusiform-elliptical, 13–20 × 5–6 μ.

Briefly, there are five distinct species in the *versipellis-scaber* complex—on the one hand, the orange-capped, cystidiate *aurantiacus* and *versipellis* (or *floccopodus*) with flesh becoming more or less roseate or violaceous, and on the other hand, the dull-colored or whitish, rarely cystidiate *scaber, leucophaeus* and *niveus* with flesh not becoming roseate or violaceous. *B. aurantiacus* has the pileus distinctly tomentose at least in part, usually entirely so, and the asperities of the stipe become reddish and finally dark brown. *B. versipellis* has a pileus glabrous or at best fibrillose-granulose under a lens, with the roughenings of the stipe becoming more blackish. *B. scaber* is glabrous, viscid, grayish, isabelline to brownish. *B. leucophaecus* is distinctly and roughly tomentose, dark brown to black. *B. niveus* is whitish with a smoother stipe than any of the other species. These species can readily be distinguished by the spore characters alone. Examination of the spores is in fact the only certain way to distinguish them (see PLATE 41), as for example, when one finds a sporophore which is quite isabelline or brownish (as of *B. scaber*) but which has a distinct orange tinge (as of *B. aurantiacus* or *B. versipellis*). These two latter species are nearly alike as to color and not easily distinguishable in this country, as they are in Europe, where the former is said to be more reddish-orange and the latter more yellow.

CERTAIN SPECIES OF THE GRANULATUS GROUP

What may be called the *granulatus* group of the Viscipelles consists of those species with exannulate, glandular-dotted stipe, even if the glandular dots are apparent only on occasional specimens. There has been a great deal of misunderstanding with regard to some of these species and difficulty has been experienced in identifying with certainty some of the others of the group.

I can offer no assistance with regard to certain of the species. I am not familiar with the western *B. flaviporus* Earle. I have not collected *B. albidipes* named by Peck, who says it is distinguished by pileus paler than that of *B. granulatus,* especially when

young, by the usual lack of dots at the top of the stipe and on the edges of the tubes (although there are occasionally a few), and by white stipe without any yellow.

Murrill made out *B. rubropunctus* Peck to be the same as *B. inflexus* Peck. I have never collected *B. inflexus,* but I have seen *B. rubropunctus* and, finding this species to be exactly as Peck described it, I am inclined to think that *B. inflexus* will be found to be distinct.

I have already pointed out that *B. placidus* and *B. brevipes* are readily distinguishable from *B. granulatus.*[5]

The other species of this group are the following: *punctipes, americanus, hirtellus* and *subaureus*—all named by Peck.

Boletus hirtellus is closely related to *B. subaureus* and I have found forms which have some of the characteristics of *B. americanus,* notably the vermilion streaks occasionally found in that species. I have also found some forms late in the fall after cold weather that had definitely boletinoid tube layers. I am reserving judgment regarding these latter forms, but in any case, the species is readily recognized by the hirtose pileus and dark color of the spore mass.

Murrill placed *B. americanus* in *B. subaureus* and *B. punctipes* in *B. granulatus.*[6] Not only is this arrangement wrong, but furthermore, the two most similar species of these forms are the middle pair—*B. subaureus* and *B. punctipes.* I have never had any trouble recognizing *B. americanus,* but I have had to learn to distinguish *B. punctipes* under certain conditions, especially before the glandular fluid of the latter becomes darkened.

Boletus americanus differs from *B. subaureus* in the following respects:—pileus more yellow (not so pale), not marked with brown by the drying gluten but with reddish streaks; pores larger and of a duller yellow color; stipe more slender, with the exuding drops whitish instead of yellow, and the flesh not reddish within at the base; the spores slightly larger; and the odor and taste usually farinaceous instead of mild and unnoticeable. *B. americanus* cannot be confused with any other species.

Boletus punctipes is nothing like *B. granulatus.* It differs in

[5] Notes on Boletes. II. Mycologia **25**: 231. 1933.

[6] Loc. cit., p. 13.

every one of the characters which are used to describe these forms —stature, color of pileus, margin, color of flesh, color of tubes, color of stipe within and without, type of glandular dots, size of spores, and cystidia. Everyone knows *B. granulatus* with pileus at first dark then pale, with flesh mostly white, with ochraceous glandular-dotted tubes and with white stipe finely dotted and yellowish above. *B. punctipes* is yellowish to cinnamon-buff on the pileus, with flesh yellowish, tubes brownish to brown, stipe *rhubarb-yellow* and thickly covered with large masses of glandular secretion that are soon brown and have a peculiar *sticky-waxy* feeling. The spores of *B. punctipes* are 9–11 × 3–4 μ, those of *B. granulatus* are mostly 6–7 × 2.5–3 μ.

Boletus subaureus and *B. punctipes* are usually readily distinguishable when mature. *B. subaureus* is always pale yellow on the pileus, the tubes are medium-sized and yellow to ochraceous, the stipe is yellow and has a more or less patchy, reddish-brown to brown glandular secretion. *B. punctipes* is yellow, but becomes cinnamon brown on the pileus, the tubes are always brownish to brown and small, the stipe is that peculiar rhubarb yellow with the closely-packed, brown, sticky-waxy-feeling glandular masses of dried secretion. No one can fail to recognize *B. punctipes* when mature.

When, however, as mentioned above, *B. punctipes* is young, with the pale-colored pileus and the pale-colored glandular dots, it is likely to be confused with *B. subaureus* in the field. When the young specimens are brought in and dried, usually the characteristic features of *B. punctipes* appear sufficiently distinctly for a diagnosis. If they do not, an examination of the spores will not fail to separate them. If all but the immature ones are over 9 μ long, it is *B. punctipes;* if all but a very few are under 9 μ long, with those few up to 10 μ long, it is *B. subaureus.* I have found this criterion infallible. *B. punctipes* also has the cystidia cylindrical to clavate. *B. subaureus* has them irregular to more or less fusiform.

THE EDULIS GROUP

Murrill included in *B. edulis* [7] (his *Ceriomyces crassus*) a number of species considered distinct by Frost and Peck. It seems to

[7] Loc. cit., p. 149.

me that most of these are good species, readily distinguished and easily recognizable.

Frost briefly described *B. limatulus* as differing from *B. edulis* only in the viscid pileus and the tube mouths yellowish-brown. *B. edulis* is often viscid or even viscose, and the tube mouths are often brownish. This is one case, therefore, in which Murrill was probably correct and this name is to be reduced to synonymy.

Frost also inadequately described another plant as *B. decorus,* differing from *B. edulis* in its tomentose to squamulose pileus, brownish-red, furfuraceous, non-reticulate stipe and tubes becoming greenish when wounded. As far as is known, no one since Frost has found this plant. This past summer, however, I found some sporophores which fit Frost's meager description in all but a few particulars. My specimens, not quite mature, were hardly tomentose on the surface, had tubes at first white and later yellow, turning rusty brown, particularly when young and white and more or less dry, and had spores a little longer than Frost's single figure. It is nothing strange to find such minor differences where the early descriptions were habitually incomplete. Frost emphasized the turning green of the tubes when wounded, but there is no way of telling whether he meant a real blue-green or a mere watery-greenish which is often assumed by crushed tubes of many species.

It appears possible that I have collected something very close to *B. decorus* and perhaps actually what Frost had, and that possibly it is a good species. I am awaiting further collections and holding the matter open. It may be added that the plants I have appear to be related to *B. eximius* and are nothing like *B. edulis.*

Murrill states that most of the other American forms [8] can be placed in Peck's varieties *clavipes,* with stipe reticulate to the base, or *separans,* with brownish-lilac cap and stipe. It appears to me that such a disposition of these forms would necessitate stretching a species concept too far. I have collected the other forms named by Peck and find them to be easily identified according to Peck's descriptions and sufficiently unlike *B. edulis* to retain their autonomy.

Boletus separans was distinguished by Peck on the basis of its adnate tubes which often separate from the stipe, its brownish-red

[8] Loc. cit., p. 149.

or brownish-lilac pileus and stipe, and strongly subacid odor while drying. The separating tubes are often noticed, but are not distinctive. The spores are very similar to those of *B. edulis,* but the stature, colors and general appearance of *B. separans* readily distinguish it.

Murrill was uncertain about including *B. auripes* in *B. edulis* and rightly so. The pileus is yellowish brown, the tubes and stipe are yellow and the flesh is yellow fading to white. These are distinctive characters.

Boletus nobilis differs from *B. edulis* in the following characters:—tubes not depressed around the stipe and without greenish tint; the flesh thin even in every large specimens, pure white except where it is yellow next to the tubes instead of more or less reddish brown; stipe white or pallid, often with a lilaceous tinge in places; and spores not so ventricose or pointed. In large troops of this species, I have occasionally found specimens with pileus more or less bleached to pure white.

Boletus Atkinsoni is distinguished by: pileus more grayish to grayish-brown than yellowish-brown or reddish-brown, with fibrillose-squamulose to rimose-squamulose surface; tube layer not much, if at all, depressed around the stipe.

Boletus variipes is very similar to *B. Atkinsoni,* but with longer and more fusiform spores.

BOLETUS CURTISII

Along with some other very interesting specimens recently sent by Mr. H. C. Beardslee was a sporophore of *B. carolinensis.* This species was described by him [9] on the assurance of another mycologist that it was distinct from *B. Curtisii* Berk. in its stipe being even instead of reticulate, as is supposed to be the case with *B. Curtisii.* Study of the specimen and of Beardslee's excellent colored photographs and notes showed that his species agrees precisely in every other particular with the usual descriptions of *B. Curtisii,* especially as to the elliptical yellow spores.

Such close agreement of the two descriptions stimulated my interest, particularly because very few of the Boletaceae possess yellow spores. I began to wonder if perhaps there had been a

[9] Jour. Elisha Mitchell Soc. **31**: 147. 1915.

mistranscription of the original description or if Berkeley had possibly made an error in his accounts of the species. Further, I remembered Peck's statement regarding *B. Curtisii*[10]—" . . . The viscose pellicle indicates a relationship to the Viscipelles, with which the reticulated stem does not harmonize. The nearly free tubes point toward the Edules against which the slender stem and unstuffed tubes militate. It is also incongruous among the Calopodes but as there seems to be no better place for it, we place it here for the present."

Returning to the original description,[11] I found that Berkeley made no mention in either the Latin or the English texts, of the stipe being reticulate. His words follow: " . . . *stipite inaequali, exannulato, cavo, stramineo;* . . . stem irregular, unequal, hollow, ringless, 2–3 inches high, ¼ of an inch thick, pale or straw-colored." On the other hand, in the later description[12] he gives the stipe as "*reticulato*." It is this later description which has been reproduced by other mycologists.

Examination of specimens of *B. Curtisii* in the New York Botanical Garden showed that the stipes are not reticulate, except possibly in places from the slightly decurrent walls of the tubes. Likewise, no sign of reticulation could be found on the stipe of the plants in the Curtis Herbarium at the Farlow Herbarium at Harvard. It is quite likely, but not certain, that these plants are cotypes.

It is clear, therefore, that *B. Curtisii* does not have a reticulate stipe and that *B. carolinensis* is the same species.

Since *B. Curtisii* does not have a reticulate stipe, it does not belong in the tribe Calopodes, where it is out of place because of its very viscose pileus, its hollow stipe and elliptical yellow spores. In which tribe to place it is somewhat of a problem.

There are three possibilities. Peck suggested the Edules because of the nearly free tubes. The tubes appear to be fundamentally adnate, but on the other hand Beardslee writes that the pores seem to be stuffed at first. Until this matter is settled, this plant cannot be put in the Edules.

[10] Boleti of the United States. Bull. N. Y. State Mus. **8**: 128. 1889.
[11] Ann. Mag. Nat. Hist. II. **12**: 429. 1853.
[12] Grevillea **1**: 35. 1872.

The second possibility is the tribe Cariosi, along with *B. castaneus, B. subalbellus,* and *B. cyanescens,* because of the hollow stipe, yellow, elliptical spores and tendency towards freedom of the tubes. The adverse considerations are the fundamentally adnate tubes, the lack of a truly oblong-elliptical shape of the spores, and the very viscose pileus. The Cariosi are decidedly dry.

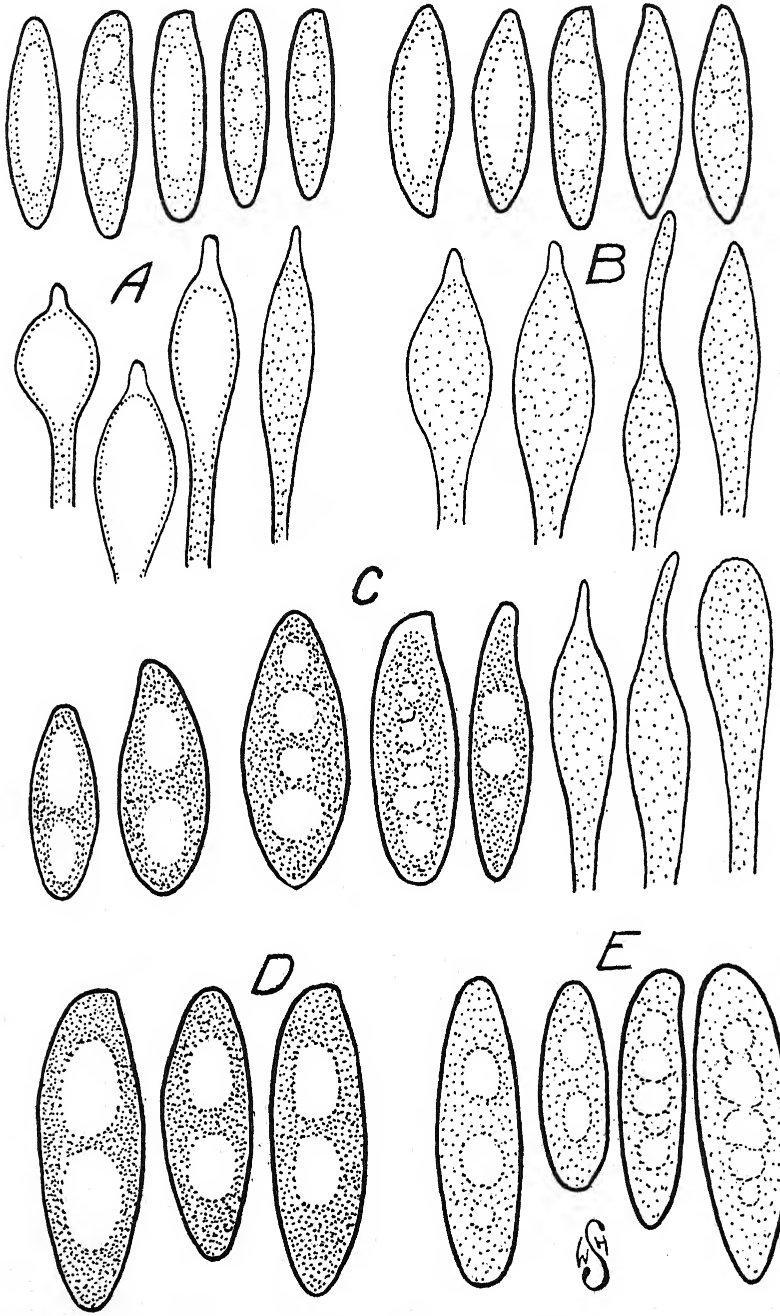
The third possibility is the Viscipelles because of the viscose pileus, adnate to decurrent tubes at least up to semi-maturity, and the elliptical spores. The characters which militate against this disposition of the species are the yellowness of the spores, and the hollow stipe. There are no species in the Viscipelles which have deep yellow spores although a few have pale yellow ones. There are no species with a truly hollow stipe. I am inclined to discount the previous reports of nearly free tubes. In Beardslee's specimens, the tubes are decidedly adnate to subdecurrent, although at the same time somewhat depressed or sinuate. The dried specimens that I have examined have the tubes much torn away for the most part, but in places still subdecurrent.

In view of these facts it seems best, on the basis of available information, to place *B. Curtisii* in the Viscipelles, while recognizing its relationship with the Cariosi.

One other point that would be of importance in settling the matter of placing *B. Curtisii* is the putrescibility or imputrescibility of the sporophore. The Cariosi are imputrescible while the Viscipelles are decidedly putrescible. Such information is at present lacking.

EELWORMS AS DESTROYERS OF FLESHY FUNGI

I suppose that it is nothing uncommon to find sporophores of the fleshy fungi in the process of rapid deterioration because of infestation by myriads of eelworms, but I am not familiar with any references to it. I first noticed that in certain localities all of the Bolete sporophores were being very rapidly reduced to a watery-slimy mess by what appeared to be a mold. The rapid deterioration of the fruit bodies was very striking. On a hand-lens examination of some specimens appearing only slightly "moldy," it was found that what appeared like hyphae slightly projecting from the surface of the entire sporophore were filamentous structures rapidly waving back and forth. These were



Spores and Cystidia of Boletus

found to be eelworms. Completely destroyed masses were a writhing mass of these minute animals.

Since that time I have examined many "moldy" fleshy forms and find that only occasionally is the "molding" of Agarics due to eelworms, but that eelworm "molding" is quite common in the Boletes. A true molding of the fleshy fungi will often result in a mummification of the fleshy sporophores, which will leave them identifiable, but it has proved very disconcerting to leave immature Boletes *in situ* for further observation, only to find that in a few hours they have collapsed to the ground in a slimy, putrid mass because of eelworm infestation.

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EXPLANATION OF PLATE 41

Spores and cystidia of the *scaber-versipellis* complex A, *B. aurantiacus;* B, *B. versipellis;* C, *B. scaber;* D, *B. leucophaeus;* E, *B. niveus.* Magnification—spores $\times$ 2000; cystidia $\times$ 1000.

A LETHAL FOR ASCUS ABORTION IN NEUROSPORA

B. O. Dodge

(WITH PLATES 42–44 AND TWO TEXT FIGURES)

Many species of fungi usually present fairly reliable diagnostic features when grown under standardized cultural conditions. Now and then, the mycelium may begin to grow out abnormally at some spot in the plate and form a sector which differs considerably from the rest of the culture in color, habit of growth, or in the amount of sporulation. An extensive literature covering the phenomenon known as "sectoring" has appeared in recent years. It is usually claimed that a mutant has been obtained because transfers from the sectored area continue to show the same abnormal type of growth, "generation after generation." In case of certain species of *Botrytis* and *Helminthosporium* where the sexual stage is unknown they were merely keeping the same individual, the same generation, alive by repeated transfers to fresh media.

Treatment of fungi with x-rays and ultraviolet light, in addition to the killing action resulting from large doses, is said to have resulted in some mutations, or in the production of ascocarps by races otherwise not ordinarily fertile. No genetic effects, however, in the cases reported, have been proved other than by vegetative propagation. *Aspergillus, Glomerella* and other forms that have been treated, can be made to produce ascocarps in culture without irradiation, but no one has adequately proved out the mutations claimed by a study of progeny obtained through sexual reproduction.

The genus *Neurospora* with its three species available for culture work affords excellent material for a study of naturally occurring mutations as well as for a study of the genetic effects obtained by treatment with x-rays or ultraviolet light. For example, when an albino non-conidial strain of *N. sitophila* appeared

during some of the writer's work, it was easy to prove that this race was in the nature of a mutant (Dodge, 1930, 1931). By mating it with a normal conidial race it was proved in every case that segregation of the factors for conidium production occurred at meiosis so that four of the ascospores in each ascus gave rise to albino non-conidial mycelia and four to mycelia which produced the orange-colored conidia normally. When two albinos were mated, all of the eight progeny ascospores in each ascus produced albino non-conidial mycelia, showing perfect Mendelian inheritance. Progeny of several generations when interbred show the same segregation, proving that the albino race arose as a mutation.

ABORTED INDURATED ASCI

Among various abnormalities that have come to the writer's notice while culturing *Neurospora* species and their hybrids, was a peculiar type of ascus abortion. Certain ascocarps produced some asci in which no spores had ever been delimited. Other asci contained normal ascospores. The aborted ascus, as it increased in size, gradually changed from a thin-walled colorless structure, to a deeply colored thick-walled body with striations resembling those normally present on the ascospores themselves (TEXT FIG. 1). These monstrosities have been found several different times; twice, in 1926 and 1929 (PLATE 42, C), in cultures of *N. tetrasperma* (strains $S_1 \times S_6$), and in 1927 in four or five cultures of F_2 hybrid origin, *N. tetrasperma* $\times$ *N. sitophila* being the original parent mating (PLATE 43, A). One such ascus was illustrated at that time (Dodge, 1928). Beyond pointing out in the legend for the plate that their nature was not understood nothing further was said. Each time they were discovered in the cultures permanent mounts were preserved, and transfers were made to fresh media to see if the abnormality carried over into the subcultures. Whether the subcultures actually developed new perithecia with aborted asci is uncertain because, in the press of other work, this important point was not followed up. It is certain that most of the subcultures failed to show ascus abortion, otherwise the cultures would have been continued. Except for four of the original cultures obtained in 1929, the cultures were finally discarded. These were preserved in their dried condition and today, now

that the subject has come up in an entirely different connection, furnish additional interesting material for genetic studies.

X-RAY TREATMENT OF ASCOSPORES AND ASCUS ABORTION

Uber and Goddard (1934), working with *Neurospora tetrasperma,* treated normal ascospores with x-rays in their studies on the killing effects of various doses. I am greatly indebted to these authors, through Dr. Goddard, for seven cultures selected at random from their series " G," cultures that had been obtained from their x-rayed ascospores. These were normally bisexual spores, but the treatment appeared to have rendered them practically, or in some cases, actually, self-sterile so far as the production of ascocarps is concerned. Testing these races for loss of sexuality by mating them against my own tester strains, S_1 and S_6, it was found that the treatment, among other effects, had largely inactivated sexually, in each case, both nuclei of the same sex originally present in the ascospore. Their strains G_4–2, G_4–4, G_5–1, and G_5–3 proved to be sex A in their reaction, while strains G_4–1, G_4–3 and G_5–1 reacted as sex B. G_4–3 and G_4–1 were thought to be actually neutral because in the first trial tests they gave no perithecia with asci when grown with either tester strain. Later tests, however, against S_6 resulted positively. They react very weakly as sex B.

One culture, G_5–3, proved most interesting. When grown alone on corn meal agar a number of large sterile perithecial bodies will develop. Occasionally one finds a few very poorly developed ascospores and one or two aborted indurated asci like those figured in plate 42. Grown against tester strain S_1, many perithecia with asci mature within ten days. In every one of the many perithecia chosen at random from seventeen different test cultures examined, there were found numbers of the aborted indurated asci mentioned above. They were mixed in with some fairly normal asci containing ascospores. Cytological studies made in 1929, to be reported later, indicated that these indurated asci were dead structures. Nevertheless, attempts to induce them to germinate were made, but without success.

Previous work on *Neurospora* had proved that in case of heterothallic races of opposite sex mated to produce perithecia, two nu-

clei, one from each of the two races, come together in each ascus and fuse. If ascus abortion here is a genetic question, that is, if the active nuclei of race G_5–3 are carrying the lethal factor responsible for ascus abortion, each ascus in every perithecium from the mating must contain such a factor, whether the ascus aborts without delimiting spores, or whether ascospores are actually matured. The mechanics by which each ascospore of *N. tetrasperma* is provided at its origin, with two nuclei of opposite sex, would provide one nucleus carrying the non-lethal element from S_1 the tester strain, and also one nucleus carrying the lethal from G_5–3. This means that every ascospore from the mating, should it germinate, would give rise to a mycelium which in turn must also produce perithecia with the usual percentage of aborted asci. Culture studies described below prove beyond a doubt that ascus abortion has a genetic basis.

Culture studies, first mating

Using race G_5–3 as one parent (sex A) and race S_1 (sex B tester) as the other parent, seventeen tube cultures on corn meal agar were started. At the end of ten days perithecia were sufficiently matured to learn that every one examined from each culture contained some aborted asci. Each perithecium always produced some mature ascospores. While normal *N. tetrasperma* does mature a few asci with other than four spores, perithecia from these (G_5–3) $\times$ S_1 cultures produced a much greater number proportionally of asci containing unisexual spores. Figure B, plate 42 shows an eight-spored ascus, four spores of which would have matured, the other four probably would have died even if they had germinated.

It should be noted here that the mycelium of the Uber and Goddard strain (G_5–3) is usually not at all normal in transfers to corn meal agar plates. Here it is often dwarfed, requiring from a week to ten days to grow over the plate. A normal mycelium would cover the plate within a day or two. Growth and branching of G_5–3 is more or less irregular and intermittent. Occasionally, however, this race tends to sector and revert to normal growth. Since the mycelium is heterokaryotic some of these irregularities may be due to the chance distribution of the four possible different

kinds of nuclei present after the x-ray treatment, a point which must be fully considered in interpreting results from irradiation. Each normal mature ascospore has four nuclei instead of two. There are two of each sex so that one nucleus of each sex could be killed outright yet if the other two nuclei in the spore had escaped injury the derived mycelium would still be bisexual and normal. Again, of the two nuclei of each sex, one in each set might be altered or killed, so that while normally there are only two kinds of nuclei, genetically, there may be four kinds after irradiation. Whether ascus abortion and dwarfing of mycelium are linked or in any way genetically connected is a question for further study. A more complete analysis of the original culture by plating out the conidia would have thrown some light on this question.

Cultures from f_1 ascospores

As noted above, the seventeen cultures from the mating (G_5–3) $\times$ S_1 developed perithecia containing some aborted asci as well as some mature ascospores. Ascospores chosen at random from different cultures were given the heat treatment. In selecting the germinated spores for transfer no particular kind was had in mind. The following spores designated by numbers were proved to be bisexual since perithecia were formed on their mycelia through self-fertilization. These perithecia produced some aborted asci as well as some mature ascospores. Nos. 1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 14, 16, 18, 19, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 33, and 38, or twenty-six in all, were bisexual. Certain ones, notably nos. 19 and 25, were very weakly bisexual. Two mycelia nos. 13 and 32 were unisexual, sex A, while nos. 7, 15, 17, and 20 were unisexual, sex B. Mycelium no. 35 continues to be very much dwarfed. It did not react sexually with either of the tester strains in the first tests. In later tests, however, it did react weakly as sex A. Three other ascospores, nos. 36, 37 and 39, failed to form mycelia after having germinated.

Perithecia from the f_1 mycelia

Having found that, of the many examined, every perithecium resulting from the mating (G_5–3) $\times$ S_1 contained some aborted in-

durated asci, it was now of great interest to see if the lethal had carried over to the next generation by means of the f_1 ascospores. At the end of about ten days a sampling was made of a few perithecia taken from each of the 26 fertile cultures listed above. Every perithecium crushed out showed some aborted asci. Races nos. 1, 2, 3, and 4 had also been grown in petri dish cultures where each matured many hundreds of perithecia. Small squares covering about 50 fruit bodies were marked off in each culture and every perithecium in each block was examined. In no case was there a failure to find aborted asci.

Perithecia from matings of mycelia from unisexual f_1 ascospores

The sex of the unisexual mycelia nos. 7, 13, 15, 17, 20, 32, and 35 had been determined by growing them separately with S_1 and S_6 as testers. Examination of the perithecia resulting from the matings showed not a single aborted ascus. Likewise when nos. 13 and 32 were mated with nos. 7, 15, 17 and 20 in pairs, the resulting perithecia all produced large numbers of normal asci, but no aborted indurated asci (PLATE 44, B). This is very easily explained if the lethal that often prevents delimitation of ascospores also inhibits germination or prevents the full development of mycelia by unisexual ascospores, even though they may germinate somewhat. A haplo-deficiency should have just this effect.

Mycelia from f_2 ascospores

Some of the ascospores that had been discharged on the petri dish covers in case of cultures nos. 1, 3 and 4, were given the heat treatment. After the spores had germinated selections were made rather roughly on the basis of size and type of germination. This would give us three classes. The larger spores would be bisexual, the smaller ones unisexual, and those with abnormal germ tubes would form a third class. The number of spores in each class has no particular significance in this case. Fifty-six spores transferred proved to be bisexual since their mycelia produced perithecia.

Of the small unisexual spores chosen eight were proved to be of sex A in their reaction and twelve were sex B. Fifteen small

spores died after having germinated. Some of them did make a mycelium a few millimeters long before dying, however. There appeared to be several grades between those that died immediately after germinating and those that made fairly normal mycelia. In general they could be classed either as abnormal (dwarfed) mycelia or as normal mycelia. Fourteen of the mycelia originally classed as more or less dwarfed are still living at this writing. They grow very slowly, but it has been proved that they do not carry the lethal for ascus abortion.

Perithecia from f_2 mycelia

A further and still more convincing proof that the lethal factor responsible for ascus abortion is segregated at meiosis is found on examining perithecia developed in the 56 cultures obtained from the bisexual f_2 ascospores. Several samples taken from each of the cultures showed aborted indurated asci in each case. No perithecium was found lacking these structures. A few of the cultures such as nos. 4.2, 4.8 and 4.13 showed weak sexuality. This led to confusion because they were at first classed as unisexual. Whenever perithecia matured, however, through self-fertilization, aborted asci were always present. Testing the unisexual races against the tester strains S_1 and S_6, and later against one another in various combinations, the perithecia resulting have given not a single aborted ascus. Race 3.31 was classed as a dwarf mycelium yet it continues to live, and it reacts very weakly with tester race S_1 to produce a few very abnormal perithecia. These contain some abnormal asci, but that they would have become wholly aborted and indurated is doubtful. It is clear that a small uninucleated spore may carry some factor for dwarfed mycelium and still be able to germinate and form a functional mycelium. Since race 3.31 mates with only S_1 tester it reacts as unisexual. In any case it would seem that the lethal action is not strong enough to entirely prevent growth and sexual reproduction.

Mycelia and perithecia from f_3 and f_4 ascospores

If one heats the ascospores for an hour at 60° C. they will usually germinate sufficiently within the next six or eight hours to

enable one to determine which ones will probably die after making a slight growth. If they do make further growth, only a dwarfed mycelium may be expected. The perithecial culture 4.11 (G_5–3) $\times$ S_1 furnished the f_3 ascospores for further testing one's ability to quickly select from spores showing short germ tubes, unisexual spores that will die soon after germinating.

Sixty-two germinating spores were selected. Of these nine were judged by their size to be bisexual, but their germ tubes appeared to be abnormal or dwarfed. Only no. 4.11.3 gave normal mycelial growth. It was unisexual, however, in its reaction. The other eight all died without making much further growth.

Eleven of the sixty-two were thought to be "rather small" and with "rather dwarfed" germ tubes. Four of these died without producing further growth, one made a dwarfed mycelium that has lived and grown slowly. Six gave rise to normal mycelial growth; only one proved to be bisexual.

Six spores were judged to be very small and "thin," but some of them had rather good germ tubes. Two of the six died without much further growth. Two gave dwarf mycelia of very slow growth, and two produced mycelia of normal growth, one of them being bisexual.

Of the seven small spores thought to be giving normal growth, six continued to grow well and one died without further growth.

It is of interest to see, without further details, that of the 49 spores judged to be germinating as dwarfs, or at least rather abnormally, 37 died after the germ tubes had added a few short branches, seven produced dwarfed mycelia of very slow growth. Only four of the whole number, 62, gave rise to fertile mycelia. This is explained by the fact, as noted previously, that the larger spores are commonly bisexual and they were, therefore, purposely avoided in this experiment, unless they appeared to be germinating very abnormally. The four exceptions were nos. 4.11.13, 4.11.18, 4.11.25, and 4.11.45. Their perithecia produced the usual percentage of aborted asci. This proves that the lethal factor was carried through the third sexual generation.

As a further test eight f_4 ascospores from culture 4.11.25 were germinated. Perithecia from each of the cultures thus obtained contained the indurated asci.

PERITHECIA IN SUBCULTURES

The lethal for aborted asci is perpetuated through the bisexual ascospores but not necessarily through a series of subcultures derived by transfers from old cultures, such as 4.11.25, after they have discharged ascospores. Fifty per cent of the small unisexual spores would be normal, and if some of them germinate after being transferred their mycelia, mating, would develop perithecia without any indurated asci. For example, two subcultures were made from each of the old cultures 4.11.18, 4.11.25, and 4.11.45. These six subcultures all developed some perithecia that did not contain any aborted asci. Other perithecia in the same cultures contained some of these structures.

NUCLEAR BEHAVIOR DURING ASCUS ABORTION

Cytological preparations made in 1929 from material representing perithecia of *N. tetrasperma* with aborted asci, and preparations made from cultures derived from the Uber and Goddard x-rayed ascospore show the same sort of nuclear degeneration. The perithecia develop to full size and form the ostiole as usual. Young asci with fusion nuclei are rather plentiful. They contain a fine granular content and take the stain deeply. Similar preparations of normal material show many examples of clear cut division figures in all three nuclear divisions. It is a very different story here. The spindle figures in aborting asci are so abnormal, no doubt due in part to improper fixations, that one cannot make out the details. Normally after the third division the eight nuclei pair up, one of each sex, giving four pairs, and each pair cuts out an ascospore. The two nuclei in the spore round up as they move back from the end of the spore, and soon after divide for the fourth time simultaneously with the nuclei of the other three spores. Every ascus of *Neurospora* then, regardless of which species is considered, at maturity contains within the spores its complement of sixteen nuclei. If an ascus contains five spores, two of them will be small and unisexual, and each of them will contain at maturity two nuclei of the same sex reaction. It would seem to be inherent in *Neurospora* for the primary fusion nucleus to undergo four successive nuclear divisions, three before, and one after, spore delimitation.

The same inheritance operates even though the fusion nucleus contains the lethal factor or deficiency for ascus abortion. In some cases the fourth division occurs immediately following the third without waiting to delimit the ascospores. The nuclei then migrate to the wall of the ascus, the spindle of the fourth division still showing between the opposite members of each pair (PLATE 44). Further study may show, however, cases of lethal action so severe as to prevent nuclear division in the ascus altogether.

The contents of the ascus degenerate but the wall begins to thicken and darken in color along branched lines so that eventually it has the same kind of striation and coloration shown by normal ascospores as they ripen, and cross sections of mature aborted asci show that the olive brown substance is deposited in just the same way (TEXT FIG. 1). About seventeen cog-like thickenings can be seen in median cross sections of ascospores of *N. tetrasperma,* whereas cross sections of aborted asci may show as many as 50 thickenings (TEXT FIG. 1). Furthermore this brittle chitinous substance is present down to the very lower end of the ascus. There are often a number of hard dark-brown spherical bodies in an aborted ascus (PLATE 42, A–C). Their nature is not clear. They are certainly not spore remnants.

Perithecia containing aborted asci often show spore abortion also. Some asci may have four good spores, but there is usually much irregularity as to their number, size and shape. As noted previously there is a greater tendency to form asci containing more than four spores than is normal for this species. Eight-spored asci are fairly common, but in non-lethal perithecia such asci are very rare. One may have to examine a thousand or more perithecia before finding one. Harper (1905) showed very clearly that in certain powdery mildews some nuclei become attached to the ascus wall and die, while the remaining nuclei delimit spores. The fact that we have in the species of *Neurospora* regularly four nuclear divisions may have some bearing on the question as to whether in certain cases of ascus abortion some of the eight nuclei may not succeed in cutting out spores while the others die. Spore abortion is very well known in many groups of Ascomycetes but cases where the ascus "aborts" but still continues to develop to become a differentiated indurated structure so that it resembles

a giant spore, have not been reported elsewhere so far as has come to the writer's attention. Zickler who treated *Sordaria macrospora* and *Neurospora crassa* with chloral hydrate obtained structures resembling our aborted indurated asci, but he did not recognize them as such.

Wodehouse (1929) has reported that in a certain variety of *Cichorium Intybus,* he finds something analogous to these indurated

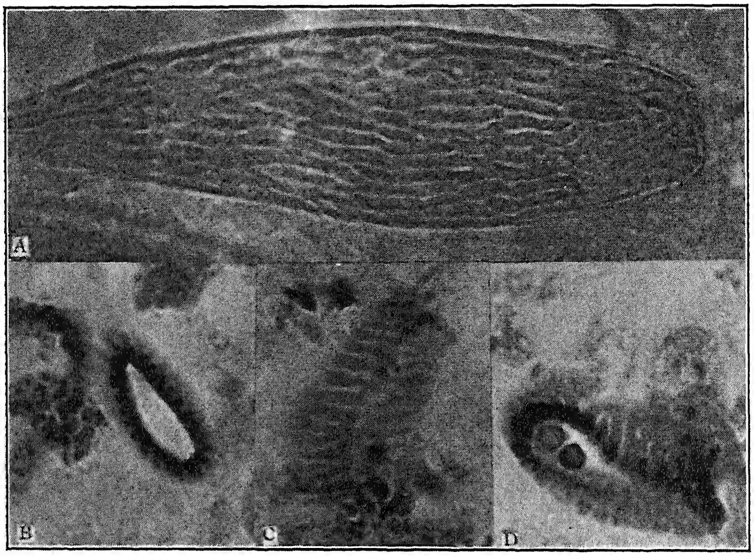

FIG. 1. A, "Aborted ascus" of *Neurospora tetrasperma,* one parent derived from an x-rayed ascospore. Such asci have thick olivaceous to dark-brown, brittle, striated walls resembling giant ascospores; B–D, Thickenings and contents as they appear in sections.

asci. He says: "For example, in the variety known as 'Red-leaved Treviso,' a large proportion of the pollen mother cells do not divide; in these the material of the special mother-cell walls is deposited in a granular concretion on the surface, but such cells appear to be deficient in power to properly organize it. . . ." "Yet, at least in some cases, spines are weakly developed, and they and the texture of the exine bear an unmistakable resemblance to those of the normal grains. The symmetrical triradiate pattern is a haptotypic character and, lacking the contact stimuli, fails to develop, but the spines and texture are emphytic and develop, at

least in part, independently of contact stimuli. These formless giants call to mind the giant spores and 'indurated asci' recently described by Dodge (1928) among the progeny of his hybrid *Neurosporas*. . . ."

In discussing this subject again with Doctor Wodehouse recently, he offered to reexamine his preparations with the view of sketching one of the abnormal pollen mother cells. The following is a quotation from his letter transmitting the sketch which is reproduced as figure 2 in the text.

"In the longitudinal sections of the buds, which were fixed just

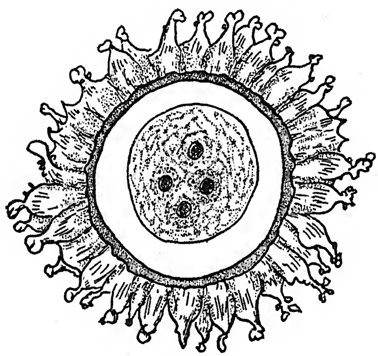

FIG. 2. "Aborted pollen mother cell" of *Cichorium Intybus* sketched by Dr. R. P. Wodehouse. Shows spine-like thickenings of mother cell wall similar to those characteristic of pollen grains. The four aborting nuclei still present. Shrinkage of the mother-cell cytoplasm due to poor fixation.

after the pollen grains reached maturity, it was found that some of the loculi contained normal pollen while adjacent loculi contained grains still united in tetrads, joined together by pit connections but otherwise almost perfectly developed, and still other loculi contained undivided pollen mother cells, apparently with one, two, three or four nuclei. In other words, in a single flower occur pollen mother cells arrested in all stages of division, from no division at all—not even the nucleus—to complete separation of the four daughter cells. But all of these, regardless of the stage of their development at the time of its arrest, received their full share of the secondary thickening material of the exine. In those grains which had succeeded in separating, this material was

properly organized, forming the normal pattern for chicory pollen which is made up of a system of high anastomosing ridges bearing pointed spines. In those which still remained joined by pit connections the pattern was more or less perfectly formed, though variously irregular. But in the undivided giants there was scarcely any suggestion of the pattern. The spines were generally more or less perfectly formed, though variously irregular in size and shape" (TEXT FIG. 2).

The markings on pollen grains are now said to be maternal in origin and inheritance. Perhaps we have in the aborted indurated asci of *Neurospora* evidence bearing on the nature of ascospore markings. No one has adequately explained how the striations to be found on the ascospores of *Ascobolus,* for example, are developed. Certainly in *Neurospora* the markings on the ascospores and those of the "aborted" ascus wall are laid down outside of the protoplast but within the outermost layer of the original wall in each case.

ABORTED INDURATED ASCUS DUE TO OTHER CAUSES

We may now consider the four dried cultures of October 26, 1929, previously mentioned as containing perithecia with many aborted indurated asci (PLATE 42, C). Those perithecia also produced some ascospores. The question arises, would cultures obtained from the ascospores have in turn produced perithecia with indurated asci and have shown a Mendelian inheritance of a lethal factor in the same way as that manifested by the progeny of the x-rayed ascospore G_5–3? To be able to answer this question five years after the cultures had dried out depended on whether the spores would still germinate.

On November 11, 1930, or about one year after the cultures had dried out, melted corn meal agar was poured into the tubes to revive the cultures. The old agar on drying had split away from the glass and curled up so that the new agar hardened below the original slant. It was easy on March 20, 1934, to separate the two slants in a tube. The perithecia that had formed on the 1930 medium contained no aborted asci, indicating that the mycelium that grew out from the revived 1929 culture was normal, just as were the mycelia that had been transferred to the tubes originally

from tester strains S_1 and S_6. Perithecia from the older slant of 1929 contained many of the deeply colored aborted asci as well as some good ascospores. Ascospores from the 1930 perithecia were given the heat treatment. They germinated abundantly as the result. Forty-four single spore cultures all produced normal perithecia and, as was to be expected, not a single aborted indurated ascus could be found in them.

Eleven perithecia containing large numbers of the brown aborted asci were then taken from the 1929 part of the culture and 148 single ascospore cultures were obtained from spores judged by their size to be bisexual. Of these 70 produced perithecia, 46 made normal growth, but when grown alone remained sterile. Thirty-two either died soon after germinating or developed very much dwarfed mycelia. A few of the fertile cultures showed much spore abortion and some evident ascus abortion but none produced the typical dark-colored aborted indurated asci.

That some lethal factor is at work to reduce the viability of the germ tubes and to inactivate the nuclei of one sex frequently is evident from the number of spores that died as soon as they had germinated or after they had developed a dwarfed mycelium, and from the number of cultures that reacted as unisexual races when they should have been bisexual.

Aborted indurated asci induced by chloral-hydrate

Zickler (1931) working with *Sordaria macrospora* and *Neurospora crassa,* treated his cultures for various periods of time with chloral-hydrate. He was able in this way to obtain numbers of giant ascospores. His figures Abt. 5 and 6, which are from photomicrographs show in addition to true giant ascospores, aborted indurated asci. In case of *N. crassa* no striations show in his reproductions but the asci are black down to the very end. He refers to these structures as giant ascospores which completely fill the ascus, lying close to the wall all around and extending down to the very bottom, taking the exact form of the ascus. If he had tried to germinate these structures or had made cytological preparations he would no doubt have found that these structures were not giant ascospores at all but really aborted indurated asci such

as the writer had figured previously (1928) and the same as are shown in our plate 42. He germinated several of the true giant ascospores but he does not report concerning progeny ascocarps nor did he claim he had affected a genotypic change.

The effect of chloral-hydrate evidently was similar to that of the unknown agent responsible for ascus abortion in our cultures of 1929, where the stimulus or destructive agent must have been active sometime during the life of the culture, so that some asci would abort while others would not be affected.

CONCLUSIONS

It is not the purpose of this paper to enter into a discussion from a genetic standpoint of the mechanism responsible for ascus abortion in the x-rayed line. Since 50% of the uninucleate f_1, f_2, or f_3, ascospores die after growing slightly, one might hazard a guess that ascus abortion is due to a typical induced deficiency comparable to that recently described by Stadler (1934) in his paper on haplo-viable deficiency in maize. Here the aborted pollen dies after the two nuclear divisions. Rarely the pollen tube emerges slightly. The deficiency is never transmitted through the male gametophyte. It is sometimes transmitted through the female gametophyte but with reduced vitality. In *Neurospora* male and female gametophytes are not differentiated as such, but they are of the plus and minus order. The deficiency, if we may call it that for convenience, is rarely, if ever, carried through a gametophyte arising from a uninucleate spore. Should a similar deficiency be induced in *N. sitophila* it would be fatal, but in *N. tetrasperma* where the ascospore will contain a normal as well as a deficient nucleus and the two nuclei divide at about the same rate, the transmission of the deficiency through the bisexual gametophyte is assured.

One can perpetuate the deficiency vegetatively if the cultures are stored at a temperature low enough to prevent the formation of ascocarps. Since the ascospores remain viable for several years one ought to obtain fresh cultures which will mature aborted indurated asci at any time by germinating the spores.

SUMMARY

A culture, G_5–3, of *Neurospora tetrasperma* derived from a bisexual x-rayed ascospore is practically self-sterile, but it reacts sexually as sex A with tester strain S_1. The resulting ascocarps contain numbers of dark-colored aborted indurated asci with no spores, as well as some asci that mature ascospores.

Cultures obtained from bisexual f_1, f_2, f_3 and f_4 ascospores produce perithecia which in turn contain the dark-colored empty asci. The lethal for ascus abortion is segregated at meiosis so that each bisexual ascospore contains at its origin one normal and one deficient nucleus, that is, one containing the lethal agent, whatever it may be. The lethal for aborted ascus is, therefore, carried on from generation to generation only through those ascospores containing two nuclei of opposite sex at their origin. The small uninucleate, and therefore unisexual ascospores are of two sorts. Some die soon after they germinate. These carry the lethal. Others being normal produce mycelia of normal growth. Of the latter, two of opposite sex, when mated together, produce normal perithecia with no aborted indurated asci whatever.

THE NEW YORK BOTANICAL GARDEN.

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EXPLANATION OF PLATES

PLATE 42. *Neurospora tetrasperma*

A. Aborted asci from mating (G_5–3) $\times S_1$. The race G_5–3 derived from an x-rayed ascospore. S_1 is a normal tester sex B race.

The lethal does not always prevent ascospore delimitation but the bisexual ascospore cut out will carry the lethal in one of its nuclei. The other nucleus will be normal.

B. Aborted asci from second generation f_2 ascospores. Note one ascus has eight spores, four larger and more mature. These would be normal unisexual spores; their mycelia when mated would give rise to normal perithecia with asci like those shown in plate 43, B. The other four spores would die even if they germinated.

C. Aborted asci from non-irradiated line, October 26, 1929. Here the ascospores, still viable in 1934, do not carry the lethal, because the agent responsible for ascus abortion was active only during ascocarp formation. The mycelium in the culture was normal, and only a certain number of asci in a fruit body were affected.

PLATE 43. *Neurospora tetrasperma*

A. Aborted asci from a line of hybrid origin. From a preparation of material obtained in 1927.

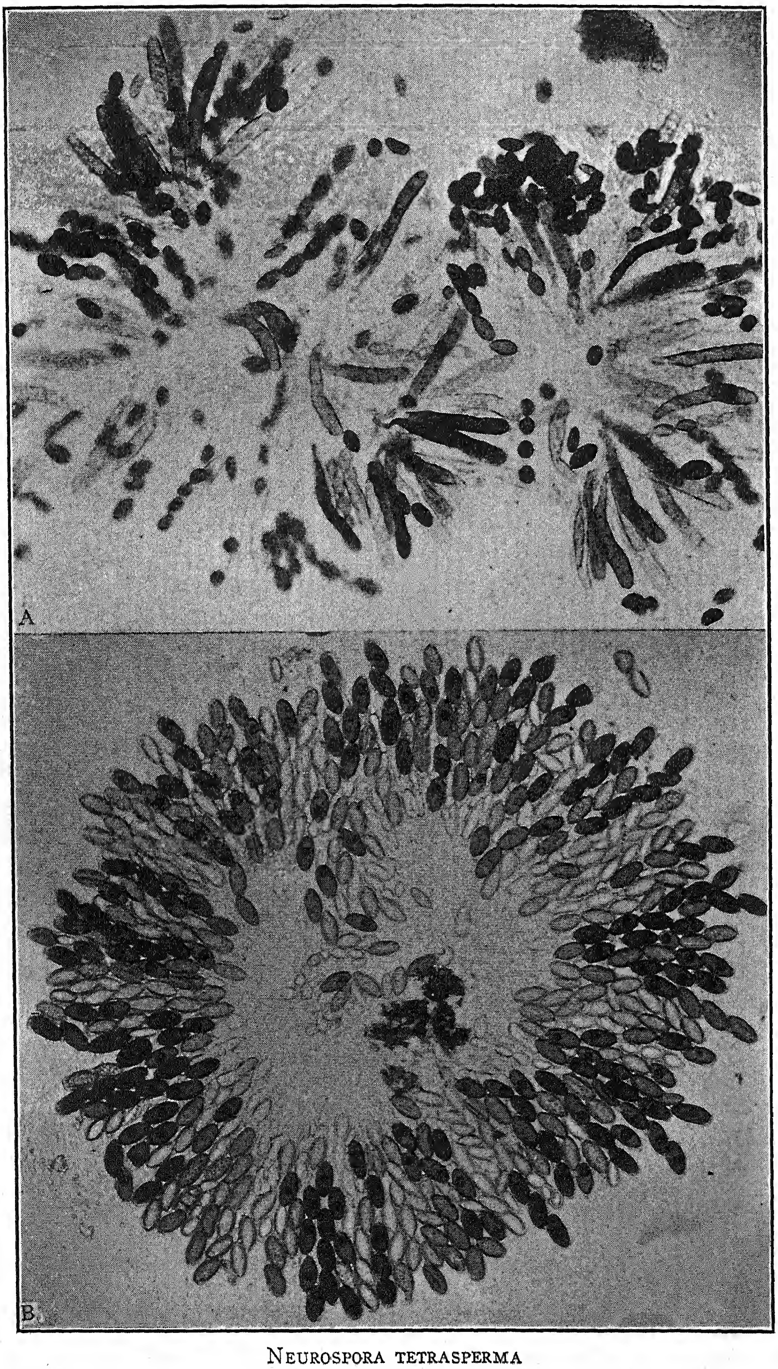
B. Normal asci from perithecium obtained by mating mycelia derived from the small unisexual spores which are not uncommon in the irradiated line.

Unisexual ascospores whose nuclei carry the lethal factors die soon after germination. "Aborted ascus" can be perpetuated through the larger spores which carry one normal and one lethal nucleus, but not through unisexual spores.

PLATE 44

Longitudinal sections of aborted asci; each of the two at the right shows seven or eight pairs of nuclei still connected by spindle fibres. The nuclei become attached to the ascus wall and die. These asci would not have formed spores.

NEUROSPORA TETRASPERMA



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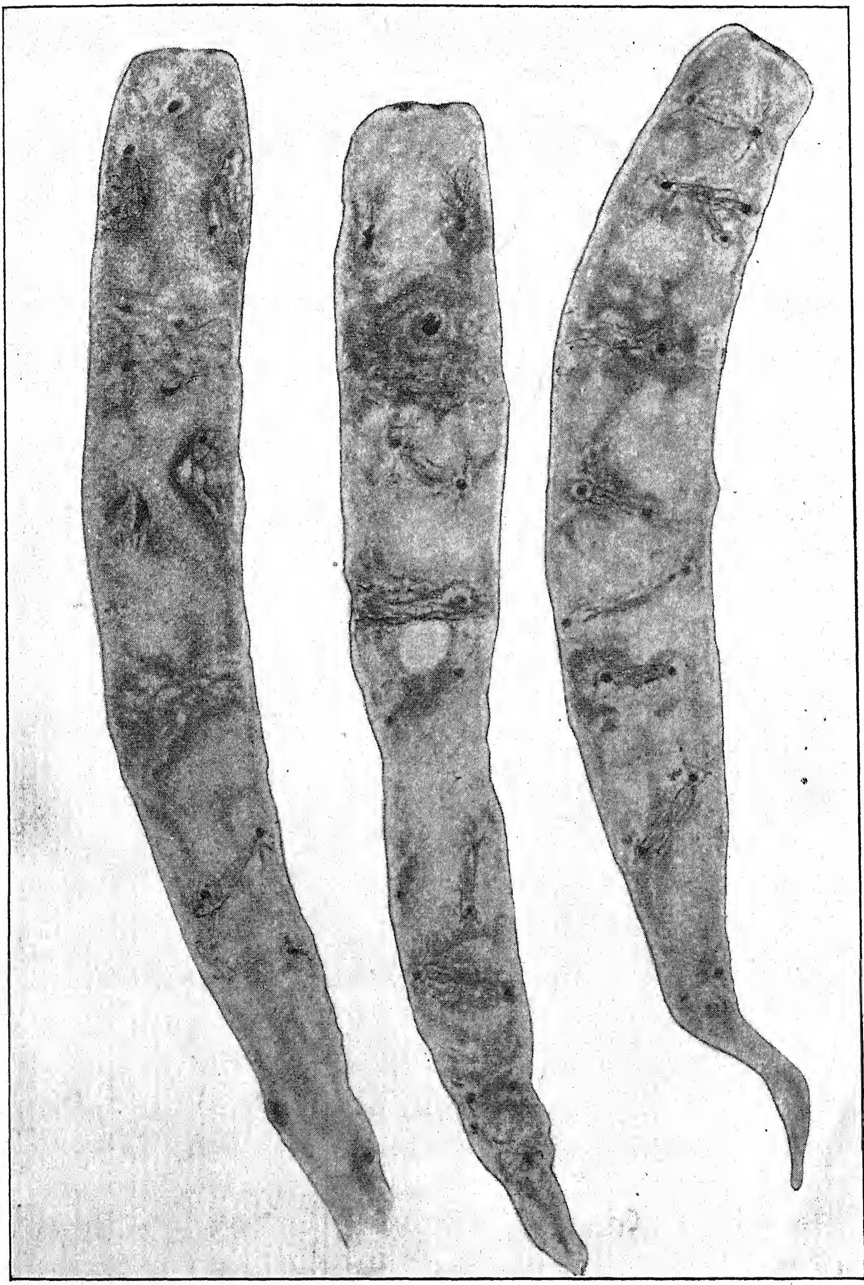

NEUROSPORA TETRASPERMA

NOTES AND BRIEF ARTICLES

Notice to Subscribers

Beginning January 1, 1935, the subscription price of MYCOLOGIA will be increased to six dollars ($6.00) per year. With this increased price it is expected that a minimum of 600 pages will be printed per year. This action will not affect members of the Mycological Society of America who will continue to receive MYCOLOGIA under the terms of the contract as formerly.

Recently, in a paper[1] containing diagnoses of a number of new chytridiaceous fungi, I applied the generic name *Scherffelia* n. gen. to a form possessing a peculiar method of development. It has come to my attention that there exists a small genus of algae belonging to the Volvocales to which this generic name has already been applied.[2] In order that Dr. Scherffel's name shall continue to be associated with this group of fungi to our knowledge of which he has contributed so many valuable observations it will be necessary to emend my first appellation of this fungus.

The following new generic name is therefore proposed for it.

Scherffeliomyces gen. nov.

Syn. *Scherffelia* Sparrow, Trans. Brit. Myc. Soc. **18** (3): 216. 1933. non *Scherffelia* Pascher, Hedwigia **52**: 281. 1912.

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DARTMOUTH COLLEGE,
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THE SEVENTH LAKE FORAY

In the previous number of MYCOLOGIA, pages 277–278, announcement was made that the summer foray of the Mycological Society will be held at Seventh Lake, near Inlet, N. Y., August

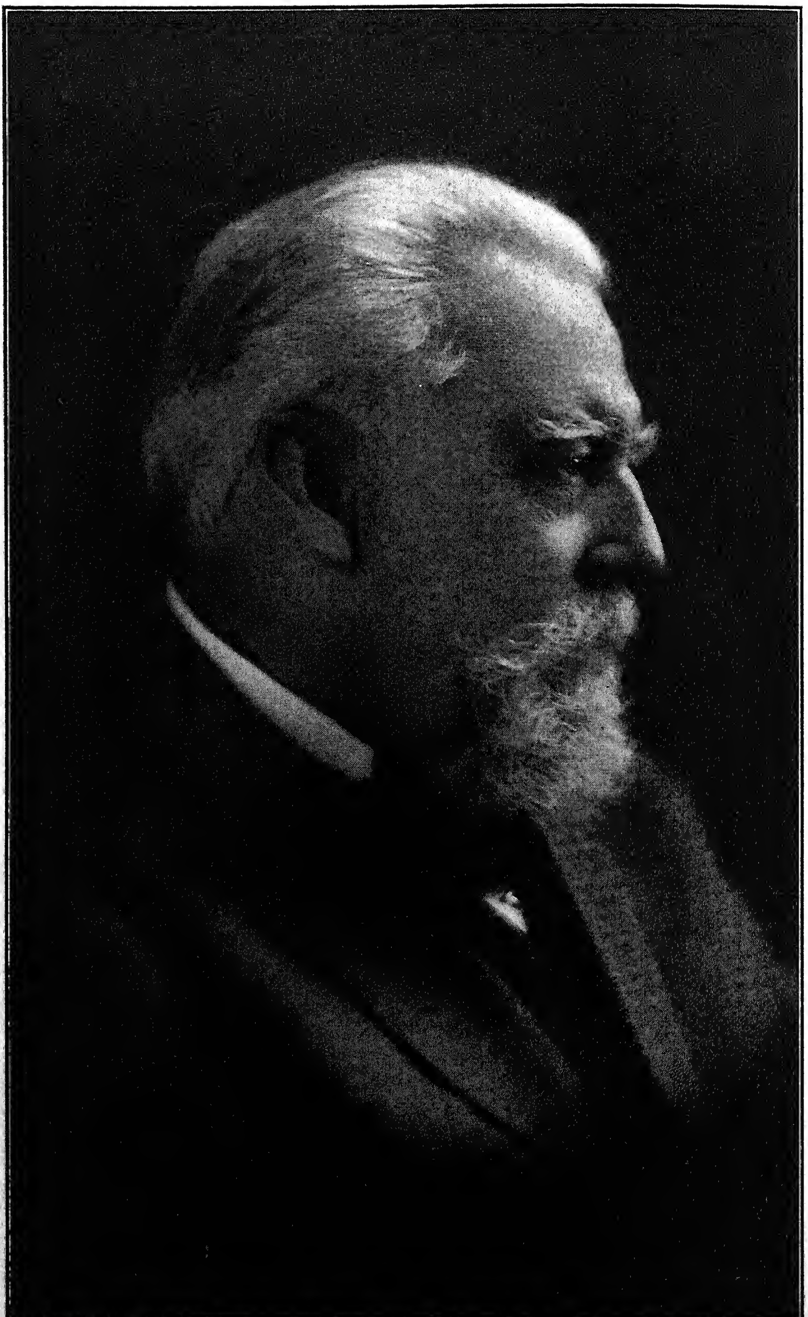
[1] Trans. Brit. Myc. Soc. **18** (3): 216. 1933.
[2] Hedwigia **52**: 281. 1912.

21–24, with headquarters at Stewart's Camp. *Mycologists expecting to attend the foray, and who have not yet made arrangements for living accommodations,* should communicate at once with Professor F. C. Stewart. Until August 10 his address will be Agricultural Experiment Station, Geneva, N. Y.; after August 10, Inlet, N. Y.

Arrangements are being made to provide lunch and dinner at Stewart's Camp thereby enabling the visiting mycologists to be together the greater part of each day. Lodging and breakfast will be provided elsewhere at three different places. Launches will take parties to Stewart's Camp each morning and return them to the lodging places in the evening. The rates, including lodging, meals, and launch service, will vary from $3 to $4 per day according to the accommodations.

Mail should be addressed in care of Stewart's Camp, Inlet, N. Y.; telegrams to Stewart's Camp on Seventh Lake, care of A. W. Every, Old Forge, N. Y. Telegraphic service is poor and expensive. *Whether arriving by train or by automobile guests should proceed at once to Every's boat landing on Seventh Lake, two miles east of Inlet, where full information may be obtained.* Motor-busses, meeting all trains at Thendara, will discharge passengers at Dad's Inn three-tenths of a mile from Every's boat landing. Motor-busses run, also, between Utica and Dad's Inn, a distance of 67 miles. Those desiring taxi service from Dad's Inn to Every's should notify A. W. Every, Inlet, N. Y., stating time of arrival.

B. O. DODGE, F. C. STEWART AND H. M. FITZPATRICK,
Committee on Arrangements



Thomas Huston Macbride

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XXVI SEPTEMBER–OCTOBER, 1934 No. 5

THOMAS HUSTON MACBRIDE

B. SHIMEK

(WITH PORTRAIT)

The death of Thomas Huston Macbride at Seattle, Washington, March 27, 1934, removes one of the last of the pioneer mycologists of the Mississippi Valley, and one of the foremost students of Myxomycetes in the world. He was connected with the State University of Iowa for fifty-six years as Professor of Botany, President and President Emeritus, and was one of the associate editors of MYCOLOGIA from its foundation to the time of its adoption by the Mycological Society.

Thomas Huston Macbride was born in Rogersville, Tennessee, July 31, 1848. His father was a Presbyterian minister of strong abolition sentiments, and the family was compelled to leave Tennessee and came to Iowa several years before the outbreak of the Civil War. They located first near Salem and later in New London, Henry County; then in Cedar Rapids, which was then a small village; and finally in Princeton, Scott County.

He received his bachelor's and master's degrees in art from Monmouth College, Illinois, in 1869 and 1873, respectively, and later received honorary degrees from Monmouth, Lenox and Coe Colleges, and from the State University of Iowa. He served as professor of mathematics and languages in Lenox College, Iowa, from 1870 to 1878, when he was called to the State University as assistant professor of natural history. His college training was

[MYCOLOGIA for July–August (**26**: 279–378) was issued August 1, 1934]

essentially in literary and linguistic fields, evidence of which was displayed throughout his life in the beauty and charm of both his written and spoken word.

His first preparation for his major life work was received on numerous field trips with his life-long friend, Dr. Samuel Calvin. The latter was a student, and also instructor in mathematics, in Lenox College, in 1864, when the former entered one of his classes in mathematics as a student. When Calvin left Lenox College in 1869 to take up his duties in the public schools of Dubuque, Iowa, Macbride succeeded him as instructor in mathematics, also teaching languages in addition. The field expeditions, which meant so much in the later activities of both men, were commenced in 1870, and continued during vacations for a number of years. These expeditions were devoted to the study of geology, botany and zoology, and on them both gained that broad, first-hand knowledge of these subjects which formed the basis for the development of the respective special fields in which both became eminent, and which gave to their teaching and research their characteristic breadth and balance.

On December 31, 1875, he married one of his former students at Lenox College, Miss Harriet Diffenderfer, who died May 28, 1927, after a happy companionship extending through more than half a century. One son, Philip D., and one daughter, Jean, survive.

In 1878 he was called to the State University of Iowa as assistant professor to Dr. Calvin, then occupying the chair (or as he later facetiously called it, the "settee") of natural history, from which were later evolved the departments of geology, botany, zoology and bacteriology. He early developed refined and advanced methods of plant histology and bacteriology when both subjects were in their infancy, and soon turned his attention to fungi and slime-moulds, which thereafter claimed his chief attention.

His first two extensive papers on fungi were published in 1888 in the "Bulletins from the Laboratories of Natural History of the State University of Iowa," then established through the efforts of Calvin and Macbride. The first of these was a report on the species of a portion of the genus *Agaricus*, then but little known in Iowa, and the second, a joint paper with Professor A. S. Hitch-

cock, on the Peronosporeae in Iowa, then equally little known. These papers were followed by others (see bibliography), but his attention was gradually drawn more and more to the Myxomycetes, on which group he became a world authority.

His first paper on slime-moulds, " The Myxomycetes of Eastern Iowa," was published in 1892, also in the University Bulletin, and attracted wide attention because the group was then almost unknown in the Mississippi Valley. This was followed by numerous other papers on the same general subject, the largest work being the standard monograph, " North American Slime-Moulds," the first edition of which appeared in 1899, the second, revised and enlarged, in 1922, and the third, which has been revised with the assistance of Dr. G. W. Martin, is in print. The collections of fungi and slime-moulds on which he based his studies are in the Herbarium of the State University of Iowa. A genus of the Hypocreales, *Macbridella,* several species of fungi and fossils, and a fern, have been named in his honor.

Macbride was a member of numerous scientific societies, and was entrusted by them with various official responsibilities. His pioneer contacts and early field experiences in primitive Iowa, coupled with his artistic temperament, early drew his attention to the need of preserving something of the original beauty and the natural wealth of his state, and he became distinctly the pioneer and the father of true conservation in Iowa. He was the founder of the Iowa Lakeside Laboratory on West Okoboji Lake, and in the past twenty-five years devoted much time and energy to the development of this inland station.

An eminent scientist, a profound scholar, an inspiring teacher, a brilliant public speaker, a public-spirited citizen, a lover of the beautiful in everything material as well as moral and ethical, a man of high character and a true friend, Dr. Macbride presented a rare combination of high qualities which made his well-rounded life an inspiration and a benediction.

His versatility is indicated by the variety of his papers and addresses. Throughout their wide range they show the influence of his early training and experience, and all are the scholarly expressions of a poetic and artistic nature. They treat of various subjects in general botany, mycology, paleobotany, geology, con-

servation and civic improvement, education, etc. Only the mycological bibliography is here presented.

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IOWA CITY, IOWA

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1934—The Myxomycetes. With the assistance of Dr. G. W. Martin.

In the earlier papers the author's name appears as "McBride," but later he returned to the original Scotch form "Macbride."

AMANITA PANTHERINA OF WESTERN WASHINGTON

J. W. Hotson and Esther Lewis

(with plates 45 and 46 and 2 text figures)

In the fall of 1912 W. A. Murrill, during a visit in western Washington, described a new species of *Amanita* as *Venenarius pantherinoides* (*Amanita pantherinoides*) (10), closely related to *Amanita pantherina* Fries. This was prior to any definite knowledge that the latter species existed in North America. It was not until 1929 that Zeller (17) reported *Amanita pantherina* Fries as fruiting in the spring in Oregon. Since then attention has been focused on both spring and fall specimens, due to their striking appearance and abundance around the Puget Sound section.

Specimens were collected in the spring and again in the fall for detailed examination and comparison. This was not difficult as there was an abundance of material available, in season, around Fort Lewis, on the Tacoma "prairies," and in the vicinity of Seattle. It has also been reported from Grays Harbor, Anacortes, Olympia, and Mount Rainier. This species is found most abundantly late in the fall from November first until the frosts come. A specimen was collected Christmas Day, 1933 in the Nisqually Valley. It commonly occurs under young Douglas firs that have abundant branches near the ground, less frequently in the open. In some instances the spring and fall collections were from under the same tree. The following is a description of *Amanita pantherina* as it occurs in the Puget Sound region.

Pileus uniform Prout's brown (15) to warm buff (15) with a darker center, sometimes yellowish, especially around the margin, covered with white persistent scales often uniformly distributed but may be nearly or completely washed off by heavy rains (Fig. 2), 5–11 cm. broad, globose when young, expanded when mature, sometimes slightly depressed in mature specimens (plate 46), viscid when moist, shiny when dry, cuticle peeling readily; *margin*

incurved, obscurely striate, remnants of the veil sometimes adhering to it (PLATE 45); *flesh* white; *gills* white when moist, creamy when dried, adnexed then free leaving a line, sometimes sinuate, up to 1 cm. broad, narrowed behind, occasionally short and truncated, sometimes floccose; *stem* 7–15 cm. long, 1–2.5 cm. thick, white when fresh, creamy when dried, equal or attenuated upward, longitudinally fibrous, floccose to scaly upward, stuffed approaching hollow, viscid when moist, bulbous, bulb subspherical measuring 2–3.5 cm. in diameter, cortex tending to peel near volva (PLATE 45); *annulus* white, bell-shaped, prominent, superior, membraneous, persistent; *volva* white, distinct, circumsessile, often curved outward, occasionally with an extra ring; *spores* hyaline, granular when immature, 1–2 oil globules nearly filling the spore when mature, 10–12.5 by 7–9 μ, elliptical, smooth, obliquely apiculate (Fig.

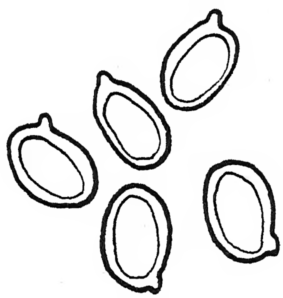

FIG. 1. The obliquely apiculate spores of *Amanita pantherina.*

1); *basidia* hyaline, 36–52 by 10–12 μ; *taste* mildly pleasant; *odor* faintly musty when fresh, somewhat nauseous when dried; *poisonous.*

This description is nearly identical with Ricken's (14) except for the gill attachment. He states they are "free" and Rea describes them as "free, reaching the stem." Both fail to mention whether or not the spores are obliquely apiculate, but this characteristic is brought out in the spore illustrations given by Bresadola (3). The latter, also, gives illustrations showing that the gills reach the stem similar to PLATE 46. Spring and fall specimens were collected and sent to Zeller who stated that both were identical with what he described as *Amanita pantherina* from Oregon.

A comparison of the above description with that of *A. panther-*

inoides shows that the shape, size and color of the pileus are practically the same. The main differences are the sinuate gills, the glabrous stem and the smaller spores. In *A. pantherina* the gills are not always sinuate but are sometimes, the stem is never glabrous when young but may become so with age. The floccose character is quite evident when the specimens are carefully handled but it may disappear by rough usage or on drying. The greatest discrepancy is in the size of the spores. For *A. pantherinoides* these are described as "ovoid, smooth, hyaline $9 \times 5\,\mu$" (10). In the species under consideration the writers have found the spores to be larger. The measuremnts given were arrived at after measuring a large number of spores from specimens collected both in the spring and in the fall and they represent the best interpretation and conception of their size although some few were found larger and others smaller. Occasionally it was found that immature spores measured as low as $9\,\mu$. As a rule these immature spores could be detected by observing the spore-content which is granular while the mature ones have 1 or 2 oil globules nearly filling the entire spore.

The writers are convinced that the spring and fall forms which are found abundantly around the Puget Sound region are identical. Furthermore, we have not been able to find forms in which the size of the spores agree with those in the description of *A. pantherinoides* although the size of individual spores have approached these measurements. The spores of a young specimen collected by Zeller and identified by Murrill as *A. pantherinoides* and deposited in the herbarium of the Botany Department of the University of Washington in 1912, were compared with those of a similar specimen of *A. pantherina* collected at Fort Lewis, November, 1933. They were found to be identical in size, shape, and content.

Closely resembling *A. pantherina* are *A. cothurnata* Atk. and the *umbrina* variety of *A. muscaria* Fries both of which probably occur in Washington. The latter may be easily recognized by the yellowish flesh beneath the cuticle of the pileus and upper part of the stem. The former, however, is not so easily distinguished. The color of the pileus of *A. cothurnata* is usually white but sometimes tinged with a citron-yellow or tawny-olive in the center. It is specimens showing the latter characteristics that are hard to dis-

tinguish from similar forms of *A. pantherina*. The final distinction is largely based on the size and shape of the spores which are described as "globose, 8–9 μ in diameter. Beardslee (1) has questioned the accuracy of this statement, claiming that "The spores, which are at first ellipsoid, lose their cell contents and become filled with a large globule as described by Atkinson, and at the same time become inflated and globose."

THE POISONOUS CONSTITUENTS

The poisonous character of *A. pantherina* has been frequently demonstrated (6). An interesting case came to our attention during November, 1933, of three persons, a man and two women, who had eaten this fungus by mistake. The following statement was given by them shortly after their recovery:

Fifteen minutes after eating the mushrooms a dizziness came on with impaired vision, evidenced by the apparent remoteness of near objects. This was followed by hysteria, then a slightly intoxicated feeling which increased rapidly, and loss of coördination of mind, speech, and muscles. The man lost consciousness within fifteen minutes after ingesting the fungus, but the women never passed beyond a semi-conscious state. In this half-awake condition, one girl imagined herself in hell and back again, although without unpleasant reactions. "In fact," she said, "it didn't appear as bad a place as some people would have us believe." The other girl imagined herself released from her position and was somewhat worried until reassured. They said they felt certain death was approaching, but were without fear. Throughout the entire illness, no suffering was felt.

In general the symptoms of mushroom poisoning as exhibited by *A. pantherina* are those of organic muscarin such as is found in *A. muscaria*. This activating principle has been isolated from this species in Japan by Inoko (4) and from the European form by Boehm (4). In order to determine whether *A. pantherina* as it occurs in the Pacific Northwest contained organic muscarin an experiment was conducted for its isolation. The procedure was the same as that followed by Brieger and reported by Blyth (2).

One hundred grams of dried fungus was ground and macerated for three days in a liter of a solution containing one per cent of

hydrogen chloride in water. Toluol was added as a preservative. This solution was then centrifuged and filtered through a Buchner filter. The filtrate was placed over a steam bath, held at 60° C., under vacuum, and the excess moisture distilled off until a syrupy substance resulted. A ten per cent mercuric chloride solution was

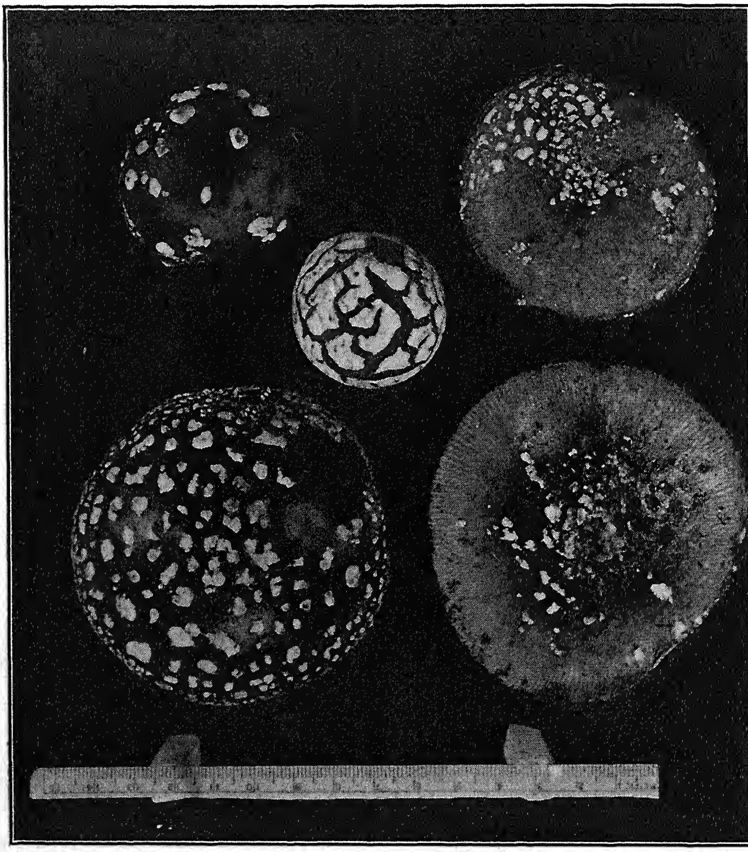

FIG. 2. *Amanita pantherina.* Showing variation in scales on the pileus.

added to this mushroom syrup, which was then centrifuged and filtered. The excess mercury was precipitated from the above filtrate by running through it an excess of hydrogen sulfide. It was again heated over a water bath of 60° C. and refitlered to remove the colloidal precipitate. A clear brown solution resulted. This was concentrated over a water bath at 60° C. under vacuum

to distill off the excess moisture. A liter of 95 per cent alcohol was added to the concentrated solution, which was then filtered, and the precipitate washed with alcohol. This was repeated using absolute alcohol. As much alcohol as possible was removed by distilling over a water bath at 35° C. under vacuum. The resulting solution, 290 cc., contained the active constituents from the 100 g. of the sample originally taken, which represents about a 34 per cent extract. Of this solution 20 cc. were taken to be used in testing for the presence of organic muscarin. To the remainder an excess of chloroplatinic acid was added and set aside to form crystals (2).

All the chemical tests made indicated the presence of organic muscarin. A precipitate was produced by auric chloride, potassio-mercuric-iodide, phosphotungstic acid and phosphomolybdic acid; but no precipitate was formed with Lugol's solution, tannic acid, chloroplatinic acid, or picric acid. Another test showed that this solution containing the active constituents was capable of reducing copper sulphate to copper oxide and ferric chloride to ferric oxide. Organic muscarin, which is colorless and strongly alkaline, is readily soluble in water and alcohol but sparingly so in chloroform and is insoluble in ether.

A further test was made to determine the effect on a frog's heart. When 1 cc. of the mushroom solution was injected subcutaneously into the back of a frog, paralysis resulted within ten minutes but no apparent action on the heart was noticed. Then 2 cc. were injected in a second frog with similar results except that paralysis occurred in three minutes. The fact that the 2 cc. contained less than .7 g. of the original active constituents would probably explain why there was no apparent heart reaction. The solution was then applied directly to the heart which stopped beating within four minutes. A one per cent solution of atropin was then applied, with no immediate effect until a weak electric current was used as a stimulant, then the heart slowly resumed its beating. Another frog was prepared and the heartbeats counted, 62 beats per minute. The extract was again applied directly to the heart and in three and a half minutes it started to miss beats; in four and a half minutes it stopped. A one per cent solution of atropin was applied, and in a few seconds it started pulsating. A weak

electric current was applied and in five minutes the heart was beating at the rate of twenty-eight beats per minute, and continued at this rate for forty-five minutes. A fourth frog was prepared and the extract applied to the heart which was arrested in diastole in four minutes; then two drops of a three per cent atropin solution were administered to the thus arrested heart. The heart slowly resumed beating without the stimualtion of a weak electric current. On the application of this mushroom solution directly to the heart the action is noticeably decreased, with lessened systolic and increased diastolic excursions, and finally stoppage in diastole. This is characteristic of the action of organic muscarin on the frog's heart (16). Large doses paralyzed the vagus and ganglia. Reflex action was perfect throughout the experiment. The conclusion is that organic muscarin is present in small amounts in *A. pantherina* of the Pacific Coast.

The writers are deeply indebted to Fred F. Johnson, assistant state chemist, for valuable assistance in extracting the muscarin; to Dr. E. Victor Smith for advice and coöperation in the experiments with the effect of muscarin on the frog's heart; and to D. E. Stuntz for valuable assistance with the photographs.

SUMMARY

1. *Amanita pantherina* Fries occurs in Washington. It has been reported from Seattle, Olympia, Mount Rainier, Fort Lewis, Tacoma, Anacortes, and Grays Harbor.
2. The writers believe that *Amanita pantherina* Fries and *A. pantherinoides* Murr. are identical.
3. Several cases of mushroom poisoning have resulted from eating this species.
4. At least one of the active principles in this mushroom is organic muscarin. This was isolated and tested.

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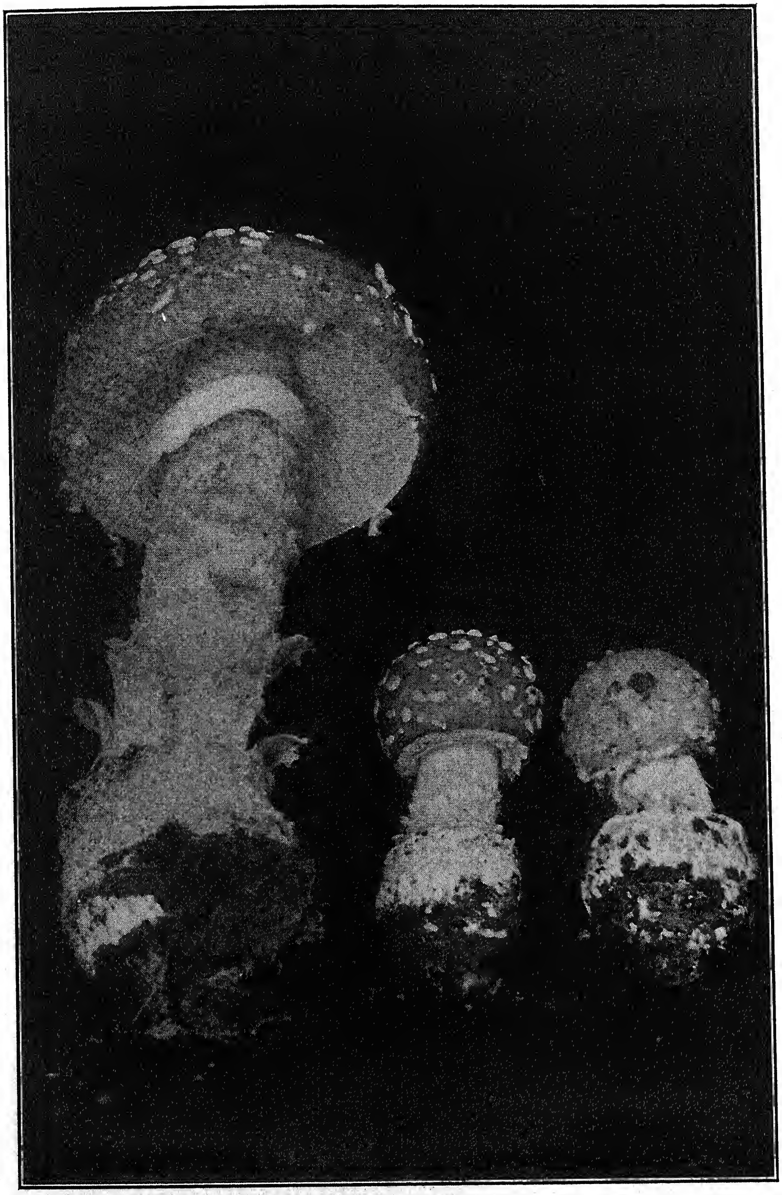
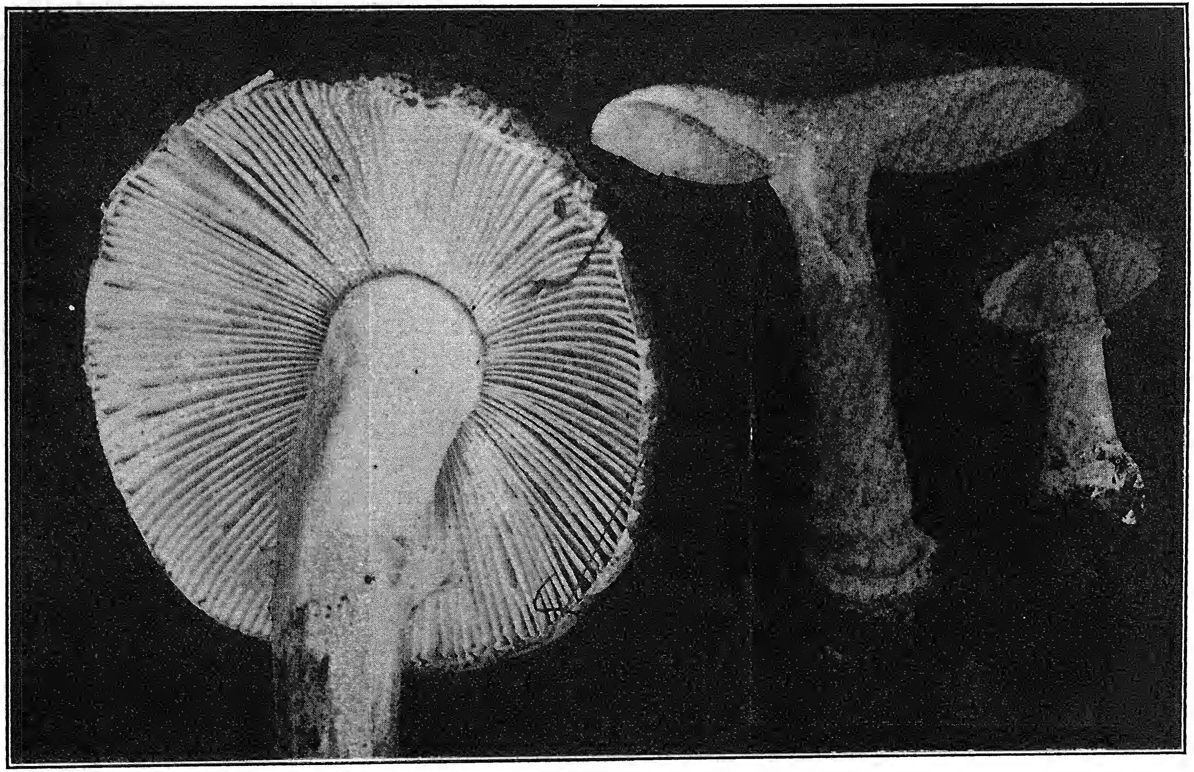


AMANITA PANTHERINA



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HERMAPHRODITISM INVOLVING SELF-STERILITY AND CROSS-FERTILITY IN THE ASCOMYCETE PLEURAGE ANSERINA [1]

L. M. AMES [2]

(WITH 6 TEXT FIGURES)

For many years the study of sexuality of fungi has been carried on with increasing interest by many mycologists and plant pathologists. By them many different sexual conditions have been discovered among the various groups of fungi, and several theories have been offered to explain these sex phenomena. Much of this work has been done in the Ascomycetes which have furnished material very suitable for such study. Recently, in *Pleurage anserina* (Ces.) Kuntze, one of the Sordariaceae, the asci of which normally contain four binucleate spores, occasionally one or two dwarf uninucleate spores and rarely one giant multinucleate spore, the writer has found that the occasional uninucleate spores when germinated developed a mycelium which is hermaphroditic and self-sterile, but reciprocally fertile with similar but compatible cultures. This situation has already been noted in a brief preliminary paper. Subsequent work has added further knowledge concerning the sexuality both of the normal, customary, binucleate spores and of the abnormal, rarely occurring, multinucleate spores. A more complete discussion concerning the sexual condition of *P. anserina* is offered in this paper.

PREVIOUS WORK ON THE SEXUALITY OF PLEURAGE ANSERINA

The development of the archicarp of *P. anserina* has been observed by Wolf (8), who found in this species (which he called

[1] Contribution from the Laboratories of Cryptogamic Botany, Harvard University, No. 131.

[2] The writer wishes to express his appreciation to Dr. Wm. H. Weston, Jr., for stimulating encouragement and helpful criticism during the progress of this investigation and the preparation of the manuscript.

Podospora anserina) that the perithecia arise from hyphal coils, and suggested that the coiling together of the two hyphae very probably represents the sexual process.

More recently, a condition of homothallism was reported for this fungus by the writer (1) as the result of experiments done during 1927–1929, with single spore cultures from the normal binucleate ascospores which occur four in an ascus. Each culture derived from a single spore, growing independently, produced many perithecia, and such a condition was interpreted to indicate that such a culture was bisexual and self-fertile (homothallic). The small uninucleate spores were observed at this time but work on them was deferred to a later date.

Subsequently Dowding (5) reported that the normal binucleate and giant multinucleate ascospores produced mycelia which were homothallic and that the mycelia from the dwarf uninucleate spores occasionally developed in the asci were heterothallic "unisexual." In the same article, she points out also that the mycelia from the normal spores bore no kind of secondary spore which might be a possible means of mixing cultures.

The writer, however, found the sexual situation in *Pleurage anserina* to be much more complex than the previous studies and reports gave evidence. In a preliminary paper (2) he showed that spermatia as well as coil-like ascogonia were produced in the cultures developed from uninucleate spores. Experimental results were reported in this preliminary paper showing that these cultures from single uninucleate spores instead of being heterothallic "unisexual," as reported by Dowding, were in reality bi-sexual and self-sterile, and that the female and male organs were morphologically distinct. Furthermore it was shown that reciprocal fertilization took place between certain of these cultures derived from the unnucleate spores; such cultures were complementary, the difference between them being not a difference of sex, because both sex organs were on each, but a difference of compatibility.

MATERIAL AND METHODS

The material of *Pleurage anserina* was first collected from horse dung at East Lansing, Michigan, in the fall of 1927, and later from the dung of various herbivorous animals in the vicinity of

Cambridge, Mass. As the cultures from the two sources are the same species and identical in all of their sexual reactions there has been no attempt in this paper to make a distinction between them.

The fungus is well adapted to the study of sexuality because the sex organs are morphologically distinct, it will develop with extreme ease in cultures, and a complete generation can be grown in approximately three weeks. The chief advantage, however, lies in the fact that within the asci which normally contain four binucleate spores, occasionally three binucleate spores and two uninucleate spores will form. These uninucleate spores enable one to obtain cultures in which there is only one type of nuclei. The importance of these uninucleate spores will be brought out later.

The media upon which *P. anserina* has been cultivated were, chiefly, potato dextrose agar and prune agar, on which the fungus grows and fruits normally. These media are of special value because, for the most part, on them the fungous mycelia remain light colored while the media remain clear, thus permitting the undisturbed ascogonia as well as the mycelia to be carefully examined with a microscope with light transmitted from beneath the Petri plate or slide. Other culture media were tried, such as dung agar, oatmeal agar, synthetic agar, etc., but because the fungus either turned them dark or would not grow well, their use was discontinued.

Single spore cultures of *P. anserina* were obtained by isolating the spores with a spatula made by grinding down a No. 10 sewing needle and inserting it in a slender handle of soft wood. After crushing a perithecium with a cover glass, the asci, as seen under the microscope, were usually spread out in a fan-like formation. If one was noticed that contained an abnormal number of spores or was for any reason desired, the cover glass was tapped carefully until that particular ascus was separated from the others. The cover glass was then slipped off and the isolated ascus allowed to become dry or almost dry. Then with the spatula, the spores were picked off in the order in which they occur in the ascus and planted separately on plates of agar. This method of obtaining single spores, besides being rapid is fairly easy.

For special study, subcultures were made on slides coated with

a thin layer of clear agar. These were prepared by pouring hot agar over sterile slides in Petri plates, so that a thin film of agar covered the slide, and the remaining agar covered the bottom of the plate and aided in keeping the culture moist and in supplying enough material for normal growth. In such subcultures from uninucleate ascospores, the sex organs as well as the mycelial growth were easily observed under a microscope while the cultures were illuminated from beneath with transmitted light.

For the working out of fertilization, the following methods were used. The sexual organs usually began to appear on these thinly coated slides about six or eight days after the inoculum had been transferred to them. After the sex organs appeared, spermatia from a compatible culture were placed upon the ascogonia either by smearing them over groups of ascogonia or by placing spermatia on particular ascogonia. The gross smears were made with a sterilized camels hair brush, on some dishes. On other dishes, the spermatia were placed on particular ascogonia with a sterilized rat whisker, used because it was flexible and therefore would not injure the delicate structures.

In the preparation of permanent slides showing the fungus in its different stages of development, cultures were flooded with killing and fixing solutions, the air was exhausted with a suction pump; the fungus was thus killed in its natural, undisturbed condition. Then the slides were removed from the Petri plate with its thin agar coat impregnated with the fungus and dealth with in a manner similar to that used for serial sections. In the latter steps, great care was needed in order to carry the film of agar through the alcohols and clearing solutions without injury. The most satisfactory stain was iron alum haematoxylin. During the process of staining it was found that even though the excess stain washed out of the agar with some difficulty, the agar became sufficiently clear to study the stained fungous parts.

GENERAL MORPHOLOGY

The details of morphology and the cultural characteristics of the fungus were determined by the study of the material in both the living and in the fixed conditions. The perithecia of *Pleurage anserina,* growing naturally on dung, may be half sunken or may

be entirely superficial, may occur scattered quite uniformly or may be aggregated in small clusters, while on agar media the perithecia range in position from totally submerged to superficial. The perithecia are membranous but not transparent, the neck being characterized by clusters of stiff, bristle-like hairs. The asci within the perithecia usually contain four binucleate spores, but occasionally asci may contain abnormal numbers of spores ranging from giant multinucleate spores to five spores, of which three are

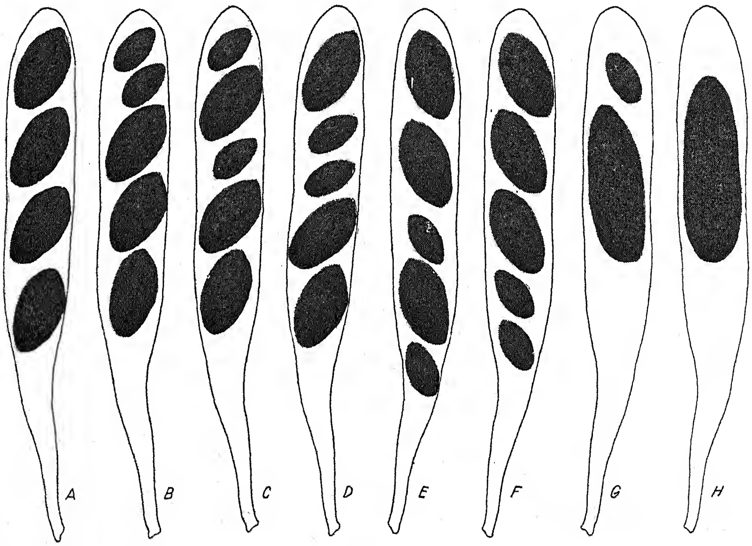

FIG. I. Diagram of asci of *Pleurage anserina* showing some variations in number and type of spores at maturity. The normal 4 spored condition (A); the condition of three normal and two small spores occasionally encountered (B–F); one large and one dwarf spore (G); and one giant spore (H).

normal binucleate and two dwarf uninucleate spores (FIG. I, B to H).

The spores of *P. anserina* are uniseriate in arrangement within the ascus, elliptical in shape, and in color range from hyaline when young through olivaceous to dark brown and opaque when mature. The normal spores range in size from 16–20 $\mu \times$ 32–42 μ, and extend below into a short, hyaline, primary appendage, 1–1½ times the length of the spore, terminated by a lash-like, gelatinous,

secondary appendage of variable length, while the apex of the spore is terminated by a similar, gelatinous appendage and in the wall at the apical end of the spore is situated a small germ pore. The formation of the spores in the ascus proceeds as follows: at first the young spore is a small allantoid cell, the contents of which do not differ materially from that of the ascus. The cell grows for a while in all dimensions, but soon all of its protoplasm congregates in and enlarges the upper end, while a septum develops separating this swollen portion, which becomes the actual spore, from the empty remainder, which becomes the primary appendage. These stages of spore formation, as observed by the writer, have been found to agree with those already described by Griffiths (**6**). The young spores in the hyaline condition, or just slightly olivaceous, are readily stained with lactophenol-cotton blue which allows the nuclei to be seen. The normal spores are binucleate, the dwarf spores are uninucleate, and the giant spores are multinucleate. This relation of spore and nuclear number in this fungus has already been noted by Wolf (1912) and discussed by Dowding (1931).

The spores germinate by the protrusion of a bulbous enlargement (FIG. II, 9), through the germ pore at the apical end; from this enlargement a few main hyphae come out, from which the mycelia arise. In general, the life cycle from beginning of mycelial growth to the formation of mature ascospores requires about three weeks at laboratory temperature. The life cycle, under most favorable conditions on dung, however, may be completed in 9 to 14 days.

The development of cultures derived from uninucleate ascospores gives an opportunity to study the morphology of the fungus and to determine the fundamental facts concerning the sex organs and their relationship to each other. Such cultures derived from uninucleate spores grow normally in every respect, but even after an interval of more than a month fail to produce perithecia. Those cultures do, however, produce both ascogonia and spermatia in abundance.

The antheridia may be solitary or associated in groups usually without regularity in position. The antheridium (FIG. III, 6, 7, 8) is usually a more or less bottle-shaped outgrowth of a cell, some-

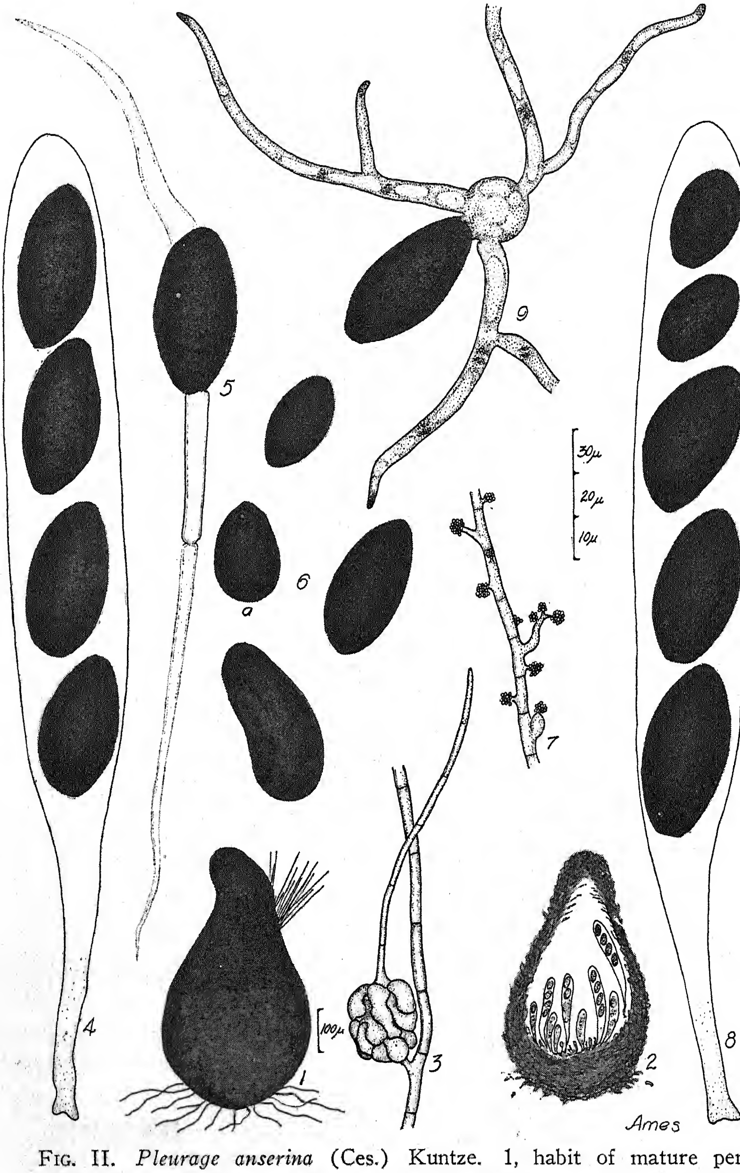

FIG. II. *Pleurage anserina* (Ces.) Kuntze. 1, habit of mature perithecium; 2, median longitudinal section of a perithecium; 3, young ascogonium with trichogyne; 4, mature ascus containing four normal binucleate ascospores; 5, ascospore showing gelatinous appendage which is characteristic of the spores of this species; 6, group of ascospores showing variation in size and shape, 6a, spore showing germ pore; 7, mycelial branch bearing antheridia with clusters of spermatia; 8, mature ascus showing three normal and two dwarf spores; 9, late stage of the germination of an ascospore.

times flask-shaped, variable in the diameter of the base, in the total length and in shape; the form and size is not constant, but the range of variation is, nevertheless, not great. In length, the antheridia range from approximately 3 μ to 5 or 8 μ; each antheridium being differentiated, more or less abruptly, into a basal somewhat broad or sometimes inflated portion (FIG. II, 7) which usually tapers to a narrow apex in which there is a short canal through which the individual spermatia are budded off successively.

The spermatia, which usually begin to appear about 6 or 8 days after ascospore germination, arise by the protrusion, through what appears to be a constriction just below the discharge pore of the antheridium, of small, uniform, uninucleate portions of protoplasm which, after emergence, becomes spherical, and the dense nucleus in each is surrounded by a transparent envelope. The spermatia are approximately 2 μ in diameter, and the enclosed nucleus measures a micron or slightly more in diameter. A single antheridium may give rise to a few or to a cluster of many spermatia. The spermatia may be transported by various means, such as air currents, water, insects, etc. The clusters of spermatia are so loosely associated that a slight disturbance may dislodge and scatter them.

The female fundaments (ascogonia) usually appear about 6 or 8 days after spore germination, and are formed from side branches on the more mature parts of the mycelium, that is, back from the rapidly growing margin of the culture; they may be submerged in the substrate or totally superficial. The ascogonia seem, for the most part, to be formed from cells which send out short, swollen branches, the contents or which are dense, and stain deeply. From observations on numerous ascogonia, it seems evident that from these enlarged, short branches others cells are produced whose contents are almost transparent in contrast with the deeply staining ones. These clear cells which in turn branch and rebranch, wrap themselves tightly about the initial dark staining cells, thus giving the appearance of a coil-like ascogonium. One of the initial branches, however, grows away from the ascogonium (FIG. II, 3), and is interpreted as a trichogyne, because it has been observed that when compatible spermatia were brought in contact with it, fertilization took place and then the ascogonium developed

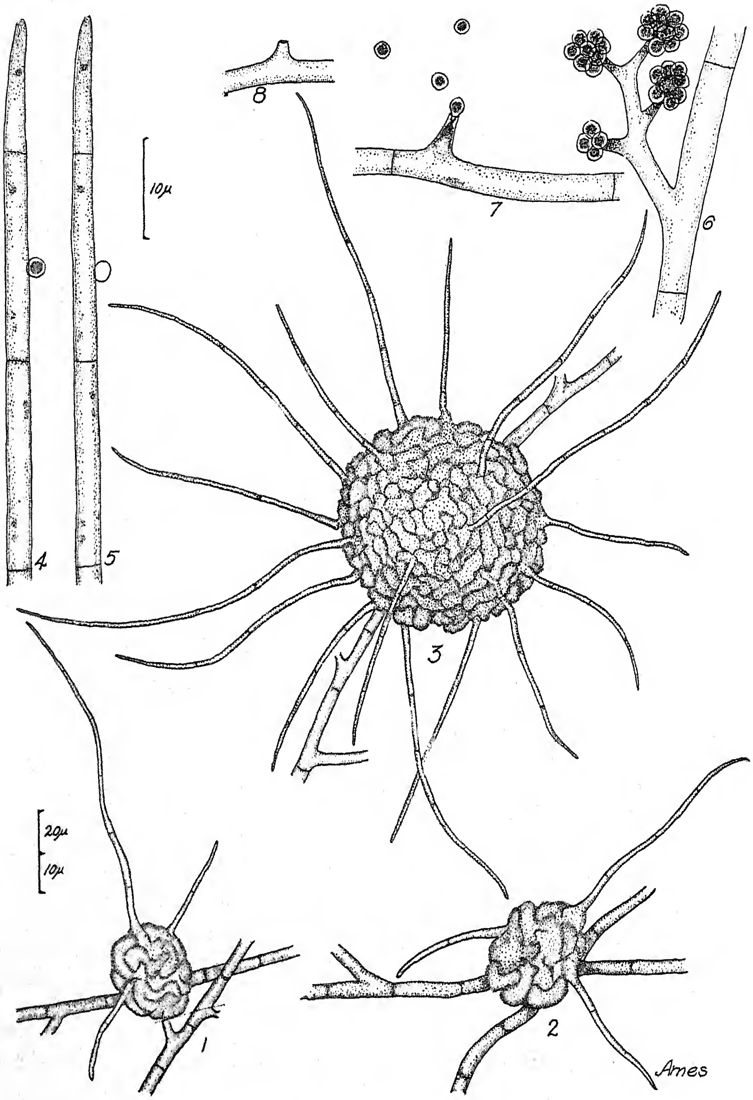

FIG. III. *Pleurage anserina* (Ces.) Kuntze. 1–3, showing different stages of development of ascogonia in which fertilization has not taken place; 4, receptive hypha showing attached spermatium; 5, receptive hypha showing an empty spermatium remainder; 6, spermatia clustered at antheridial tips; 7, emergence of a spermatium from an antheridium; 8, empty antheridium showing aperature.

into a perithecium. Very often radiating from the young ascogonium there are secondary receptive branches which also function as trichogynes. At this stage of development, the ascogonium normally becomes fertilized. If fertilization does not occur, however, the ascogonium slowly increase in size, sometimes to as much as 50 or 75 μ in diameter, and at the same time send out more receptive branches until, in some cases, these may increase to as many

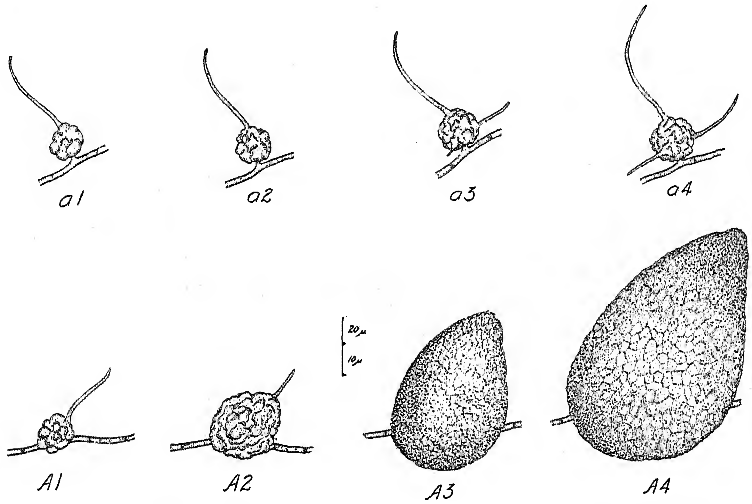

FIG. IV. *Pleurage anserina* (Ces.) Kuntze. Drawings made by aid of camera lucida showing stages in the development of two ascogonia. a, ascogonium in which fertilization has not taken place, A, ascogonium in which fertilization has taken place.

a2, A2 at the end of 36 hours.
a3, A3 at the end of 72 hours.
a4, A4 at the end of 96 hours.

as 40 or 50 in number (FIG. III, 3). The receptive hyphae are composed of a variable number of cells, usually from 2 or 3 to 8 or 10 cells. If fertilization does take place in an ascogonium it develops rapidly into an organized parenchymatous structure, spherical at first but soon forming a neck due to unequal growth at the apex (FIG. IV); the perithecia are pear shape at maturity, and bear on the convex surface (FIG. II, 1) of the neck tufts of long dark brown, sparsely septate hairs.

EXPERIMENTAL WORK

The formation of perithecia in *Pleurage anserina* involves a situation more complicated than might have been assumed from previous work reported for this and for other ascomycetes. In the cultures from uninucleate spores the condition of hermaphroditism was first suspected in the fall of 1930, as the result of an accident. In one Petri dish two complementary mycelial cultures were growing, each culture was derived from a uninucleate ascospore, and a line of young perithecia had already formed along the margin of their intermingling hyphae; at this stage the writer noticed a small mite crawling on one mycelium and in spite of the fact the plate was considered spoiled the writer, through curiosity, watched the progress of the mite's wanderings from day to day as it made a tortuous path from one mycelium over the other. After several days it was noticed that perithecia were beginning to grow in the path made by the mite, and in two weeks there was no question of this because the tortuous trail was well defined by mature and maturing perithecia. This observation led to the assumption that the mite had transported something on its body from one mycelium to the other. A microscopic study disclosed that in addition to coil-like ascogonia from which, after fertilization, the perithecia develop, there were produced on short branches minute spores which looked suspiciously as if they might be functional spermatia. This suggested that it was probably these minute bodies rather than bits of mycelium which had been transported by the mite, and that even the line of perithecia along the intermingling margin of the two mycelia probably had resulted from the fertilization of the ascogonia by these bodies rather than by hyphae. Following the clue suggested from the results of observation of the mite, and from preliminary microscopic observations of the fungus, many more single uninucleate ascospore cultures were examined, and each was found to produce both ascogonia and spermatia; yet these cultures derived from uninucleate spores never, independently, produced perithecia, even though both the ascogonia and spermatia were present, and even though many were in contact which thereby provided ample opportunity for self-fertilization. It seemed probable, therefore, that the asco-

gonia and microspores were, respectively, funtional female and functional male bodies, but that on the same mycelium they were self-sterile, while from one mycelium to another they would be cross-fertile. Accordingly tests were made to settle this point.

When, between complementary cultures, spermatia of one were transferred to the ascogonia of the second, and spermatia of the second were transferred to the ascogonia of the first, fertilization took place and perithecia were developed on both cultures (FIG. V). Reciprocal crosses have been made using innumerable spermatia streaked over many ascogonia, and reciprocal crosses have been made in which one particular ascogonium in each culture was under observation; fruiting bodies developed only from those ascogonia that had been fertilized with spermatia from complementary cultures.

Between 250 and 300 single uninucleate spores were isolated and the cultures derived from them studied; these were found to fall into two classes, which can be designated as class *A* and class *B*, in a ratio of 1 : 1. All cultures derived from single uninucleate spores belonging to class *A* are self-sterile and reciprocally inter-sterile; all cultures derived from single uninucleate spores belonging to class *B* are self-sterile and reciprocally inter-sterile; but any one or all cultures derived from single uninucleate spores of class *A* are reciprocally fertile with any one or all cultures derived from single uninucleate spores of class B. From the many experiments performed and the microscopic examinations made, it was now clear that the difference between the cultures derived from uninucleate ascospores designated above as class *A* and class *B* is not a difference of sex but represents a difference of compatibility.

It seemed imperative at this time to find out specifically how fertilization was initiated and what part or parts of the fungus entered into the sexual act. Accordingly the following experiments were performed. In one case a compatible spermatium was placed in contact with a primary receptive hypha, three cells from its tip, of a very small ascogonium which had not produced secondary receptive hyphae, and whereas the spermatium was observed to be filled with dense contents when first in contact, later the spermatium was found to be empty. It is assumed, therefore, that even though the actual migration of the spermatium nucleus

has not been observed, the nucleus passed down the receptive hypha and reached the essential female cell, because the ascogonium promptly developed into a perithecium with normal asci and ascospores. In another instance the spermatium was placed two cells distant from the ascogonium, in contact with the primary receptive hypha; the spermatium was observed to be filled with dense contents when placed in contact with the receptive hypha, and later when observed, the spermatium was found to be empty; the ascogonium in a few days developed into a mature perithecium. In still another case, spermatia were placed on the coil-like portion of the ascogonium; the contents of the spermatia could not be observed in this instance, but fertilization must have taken place because of the prompt development of the ascogonium into a mature perithecium. Compatible spermatia have been placed in contact with the mycelium adjacent to the ascogonium in several cases, but the spermatia remained the same, retaining their dense contents and the ascogonia did not develop. Ascogonia from cultures derived from uninucleate spores have had mycelia from a compatible culture in contact with their receptive hyphae, but no evidence of fertilization followed. In the same way, when compatible spermatia were placed in contact with one another, no fusion nor growth was seen to occur; and likewise compatible ascogonia in contact did not initiate fertilization.

Spermatia from incompatible cultures were also brought in contact with mycelia in the attempt to cause fertilization, but the spermatia lost none of their contents showing their inability to effect fertilization and as might be expected no perithecia developed. Likewise in any one culture from a uninucleate spore, the ascogonia were in no way affected by the spermatia from the same or other incompatible cultures. The spermatia from this source, when in contact with receptive hyphae of the ascogonia, remained unchanged, and the ascogonia did not develop into perithecia.

The male and female organs from any one culture derived from a uninucleate spore, were, therefore, shown to be incompatible, and further it was shown that fertilization takes place only when spermatia and ascogonia from separate but compatible cultures come in contact. It has therefore been concluded that cultures derived from uninucleate spores are structurally hermaphroditic, in that

they produce both male and female organs, but the behavior of these male and female organs gives evidence that, although they are self-sterile they are also each functional, and that there resides in the nuclei of these uninucleate spores the genetical qualities involving a difference of compatibility instead of a difference of sex.

Although it has not been possible, as yet, to trace the migration of the male nucleus, or to determine cytologically the part it plays in fertilization yet some points may be assumed from the experimental evidence. If, as some might argue, the spermatium nucleus plays no part in effecting fertilization, and if this process is accomplished through the fusion of various nuclei originating in the ascogonium, then we would expect to find production of perithecia in cultures derived from uninucleate ascospores. As already stated, however, the experiments demonstrate that such cultures are self-sterile and never do produce perithecia. From the experiments, also, we find that in these cultures from uninucleate spores, two classes of compatibility are involved. The production of perithecia follows the bringing together of two such compatible cultures. Furthermore experimental evidence indicates that these qualities of compatibility are inherent in the nuclei. If then, the spermatia from a culture of one compatibility do indeed take part in the fertilization of the ascogonia of the culture of the other compatibility, we would expect to find in the distribution of the nuclei in the ascospores of the perithecia that develop after this fertilization, a segregation of the two classes of compatibility in a ratio of 1 : 1. The experiments which have been carried out indicate that this is indeed the case. It seems justifiable, therefore, to assume that fertilization is accomplished by the fusion of ascogonial with spermatial nuclei, and not by the fusion of paired ascogonial nuclei.

After working out the sexual situation of the cultures derived from the uninucleate ascospores, however, and finding that they fall into two groups which differ from each other, not in sex, but in compatibility, and after having assumed that the quality of compatibility resides in the nucleus and is segregated in a ratio of 1 : 1, the writer was led to the question of the status of the normal binucleate spores. Previously the binucleate spore cultures of *P. anserina* were reported by Ames (1930) and by Dowding (1931)

to be homothallic because the mycelium from a single spore produced perithecia. It was assumed at that time that the mycelia from such single spore cultures were necessarily bisexual and self-fertile (homothallic), because they produced fruiting bodies when grown alone.

From the behavior of the cutlures from the uninucleate ascospores, it seemed possible to the writer that the mycelia produced from the normal binucleate ascospores were, in reality, not homothallic, *i.e.,* simply bisexual and self-fertile, as they were previously thought to be, but gave only the appearance of homothallism. Experiments were carried on to see if this were true, with the following results.

If the binucleate ascospores really contain two compatible nuclei, it seemed possible that by isolating many hyphal tips some of them would contain nuclei of only one class of compatibility. As a means of studying the sexual condition of the normal binucleate ascospores, some of the spores were isolated on agar, and shortly after germination had begun, hyphal tips were isolated. By this means six asci were separated from three perithecia and of the 24 spores isolated twenty germinated. From the mycelia derived from these twenty ascospores, 207 isolations were made. Those isolations fell into three groups; in the first group *A,* 23 isolations which, when grown by themselves or grown among themselves, remained without fruiting; in the second group *B* there were 19 isolations, which, when grown by themselves or grown among themselves, remained without fruiting; but any one or all of the first group A, were reciprocally fertile with any one or all of the second group *B*. The crossing experiments, as well as the microscopic examinations, show that the two groups, *A* and *B,* are each hermaphroditic and self-sterile, similar in every respect to the two groups derived from the uninucleate spores with which they were further tested. In the third group, *C,* there were 165 isolations which, when grown separately, produced perithecia. It was obvious that in each isolation of group *C* there must have been present nuclei of both types of compatibility.

On the basis that two compatible ascospores differ from each other only in respect to compatibility, and on the basis that the genetical quality of compatibility resides in their nuclei, it is as-

sumed, therefore, that the two nuclei in the normal ascospores differ from each other, not in sex, but in respect to compatibility. This assumption is borne out by the fact, as has already been shown, that it is possible to separate these two types of nuclei, from the mycelium derived from germinated binucleate spores, by means of hyphal tip isolations. The cultures derived from the normal binucleate ascospores of *P. anserina,* therefore, only appear

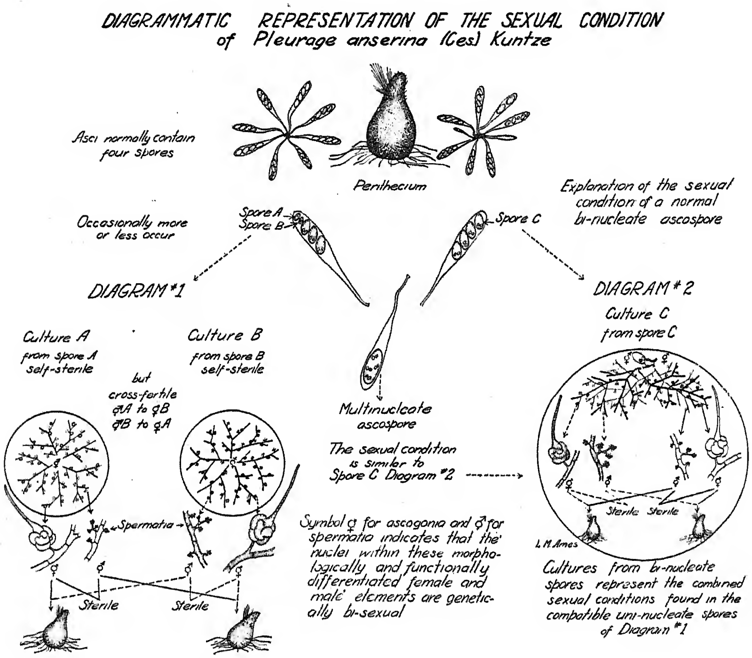

FIG. V. *Pleurage anserina* (Ces.) Kuntze. Diagrammatic representation of the sexual condition in the hermaphroditic self-sterile cultures derived from the uninucleate ascospores A and B of *P. anserina,* and the production of perithecia by cross-fertilization of compatible strains. Diagram C of culture C shows the sexual condition of mycelia derived from normal binucleate ascospores.

to be homothallic, that is hermaphroditic and self-fertile; but in reality, as the data disclose, there are two hermaphroditic cultures present growing intermingled as one, each giving rise to both male and female organs which are self-sterile, but which are reciprocally fertile (FIG. V). This condition is quite different from that defined as homothallism.

In the case of the giant multinucleate ascospores also the sexual situation was found to be essentially like that described for the normal binucleate ascospores, except that more initial nuclei are concerned, eight nuclei being enclosed in a single giant ascospore. The sexual nature of the mycelia derived from the giant spores was studied in a way similar to that just described for the normal spores. Three multinucleate ascospores were isolated, and, shortly after germination, 62 hyphal tips were cut off and transplanted to separate plates. The development of the cultures from the hyphal tip isolations showed that they also fell into three groups. In the first group A, there were six isolations which, when grown by themselves or grown among themselves, remained without producing perithecia; in the second group *B*, there were eight isolations, which, when grown by themselves or grown among themselves, remained without producing perithecia; but any one or all cultures of the first group *A*, were reciprocally fertile with any one or all of the second group *B*. The crossing experiments, as well as microscopic examinations, show that the two groups, *A* and *B*, are each hermaphroditic and self-sterile, similar in every respect to the two groups derived from the uninucleate spores with which they were further tested. In the third group *C*, there were 48 isolations which, when grown separately, produced perithecia. It is apparent that in each isolation of group *C*, there must have been present nuclei of both types of compatibility.

In contrast to the usual distribution of nuclei in the ascospores of *P. anserina*, there are occasionally exceptional cases of nuclear distribution. From the study of the normal binucleate ascospores thus far discussed it would be expected that the mycelia from all binucleate ascospores will produce perithecia when grown by themselves. From the diagram (FIG. VI, *A*) it is seen that all normal ascospores contain two nuclei which are compatible; occasionally, however, the nuclear distribution may vary, since it was found to be possible for two nuclei which are of the same compatibility class, and therefore inter-sterile, to be enclosed in a single, apparently normal, ascospore. Because the nuclei are of the same compatibility class, the ascogonia and spermatia produced by the mycelium of such a spore will be self-sterile.

Of all the binucleate ascospores grown, the writer has encoun-

tered only one case in which the mycelium from a binucleate ascospore failed to produce perithecia when grown by itself. In this one case, the ascus studied had three binucleate spores and two

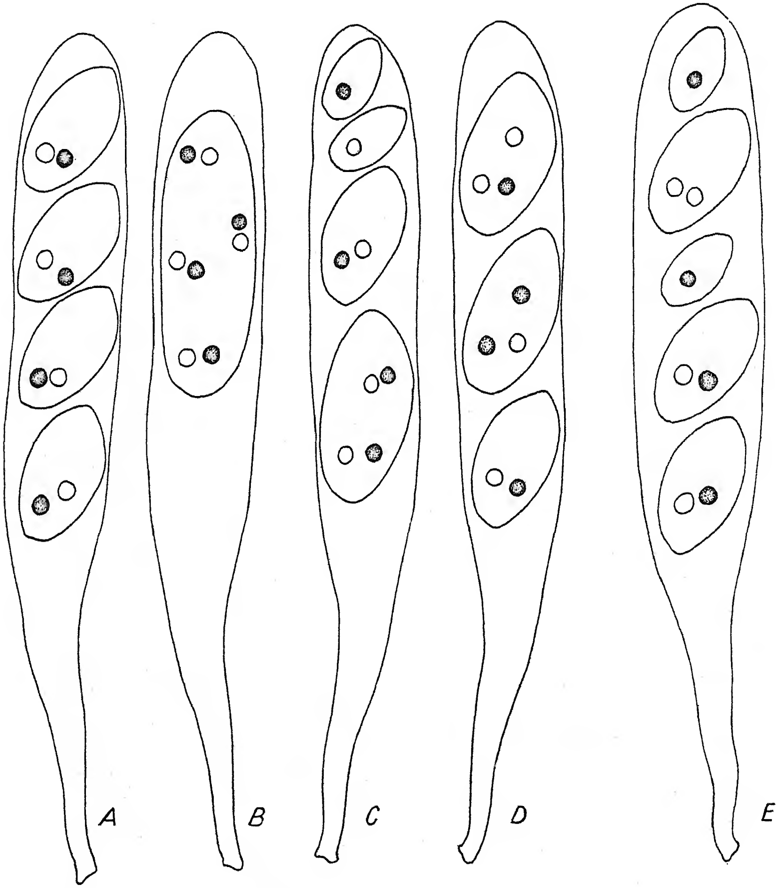

FIG. VI. *Pleurage anserina* (Ces.) Kuntze. Diagram of asci with various numbers of spores showing their respective nuclear condition. A, normal binucleate spores; B, C, D, nuclear distribution in abnormal spores; E, an unusual case in which an ascus contains two normal binucleate spores, one binucleate spore containing two nuclei of the same compatibility, and two uninucleate spores of the same compatibility.

dwarf spores, arranged from stipe to apex (FIG. VI, *E*), thus: two binucleate spores, one dwarf spore, one binucleate spore, and the final dwarf spore. These were picked off in the order of occur-

rence and grown separately on agar. The binucleate ascospore which formed between the two dwarf ones, grew normally in every way, produced abundant ascogonia and spermatia, but never produced perithecia. The mycelia from the two dwarf spores, when mated together, did not produce perithecia. However, reciprocal crosses between either of the cultures from the dwarf spores with the culture from the binucleate spore resulted in the production of perithecia. This shows that the nuclei must have been arranged so that this one spore enclosed two nuclei of the same compatibility class, and therefore was inter-sterile, and that each of the dwarf spores contained a nucleus of the same compatibility class, and were likewise inter-sterile. The other two binucleate ascospores gave rise to cultures each of which produced perithecia when grown by themselves, and hence contained the normal distribution of compatible nuclei. The segregation of nuclei in the development of the ascospores of *P. anserina* may probably be explained in the same way that Dodge (4) has shown for the nuclei of *Neurospora tetrasperma,* except that instead of a segregation of sex as shown for *Neurospora,* there is in *Pleurage anserina* a segregation of compatibility; the two types of nuclei may be segregated equally well in the first, second, or third divisions and become arranged in such a manner that each normal spore will contain a pair of compatible nuclei.

The experimental evidence shows that the ascospore nuclei of *P. anserina* fall into two distinct classes, *A* and *B*, in a ratio of 1:1. The nuclei in the mycelium of each class have the power to initiate the formation of both ascogonia and spermatia which, even though self-sterile, are nevertheless each functional. It is seen that each class does not represent a difference of sex, but represents a difference of compatibility.

Because it has been found that cultures from uninucleate spores of *P. anserina* are bisexual self-sterile but cross-fertile, it is no longer justifiable to conclude that a culture from a single spore is unisexual simply because it does not produce fruiting bodies when grown by itself and because fruiting bodies are initiated by mating complementary cultures; likewise it is no longer justifiable to conclude that a culture is simply bisexual and self-fertile because it does produce fruiting bodies when grown by itself.

It has been noted in a general way, no accurate counts being made, that the relative number of ascogonia formed on cultures from class *A* are approximately equal in number to the ascogonia formed on cultures from class *B*. Likewise the same relative number of antheridia formed on cultures of class *A* and *B* are approximately equal, thus there is an equal chance for reciprocal fertilization.

It has previously been pointed out that cultures of class *A* and *B* are each hermaphroditic; but there is also evidence that within the nuclei of the male and female organs on the mycelia from each class are the genetical qualities of bisexuality. Previous investigators in various fields have demonstrated that male and female structures contain such qualities.

That male and female organs carry bisexual genetical factors has been clearly demonstrated in *Vaucheria hamata* (Vauch.) D.C., by Wettstein (7); who demonstrated that the sexual organs of this plant have the ability each to regenerate and form a bisexual plant. He has shown that the contents of an antheridium may form a thallus which is bisexual and in every way similar to the original plant, and in the same way an oogonium may regenerate a bisexual plant.

In the fungi, Couch (3) has germinated an egg from an oogonium of Dictyuchus and the thallus from this germinated egg showed that the antheridial nature, although normally latent, was present nevertheless and could be brought out under certain conditions.

It has not been possible, as yet, to germinate the spermatia of *Pleurage anserina* nor to regenerate a mycelium from an ascogonium and thereby demonstrate their bisexual condition directly, in a way similar to that described for *Vaucheria* by Wettstein and for *Dictyuchus* by Couch.

The bisexual qualities of the nuclei in the spermatia and ascogonia can be assumed from experiments showing that after fertilization there is, at reduction division in the asci, a segregation of compatibility factors such that four nuclei are of one compatibility and four of a complementary compatibility; but there is no segregation of sex, all haploid nuclei are genetically bisexual. This is brought out clearly when haploid uninucleate ascospores are germinated and their mycelia produce both male and female

organs. Mycelial cultures derived from haploid uninucleate ascospores fall into two groups which have been designated as belonging to class *A* and class *B* which differ, not in sex, but in respect to compatibility. If we represent this difference of compatibility of class *A* and *B* by two factors S_1 and S_2 respectively we may assume from the experimental evidence that factor S_1 and S_2 are allelomorphic and that associated with each are sex factors. Thus, for example, a haploid uninucleate spore belonging to class *B* carrying factor S_2 gives rise to a mycelium in which all the nuclei carry factor S_2, consequently at the period of maturity during which male and female organs are formed, it seems to be a matter of equal possibility for production of male or female organs, caused by non-gene influence, that determines whether a cell of the mycelium will develop either male or female organs. Thus in cultures of class *B*, derived from haploid uninucleate spores, all the male and female elements contain in each of their nuclei the genetical factor S_2 and the genetical qualities of bisexuality. Likewise in the cultures of class *A*, derived from haploid uninucleate ascospores, all the male and female elements contain in each of their nuclei the genetical factor S_1 and the genetical qualities of bisexuality.

When an ascogonium of class *A* whose nuclei contain factor S_1 is fertilized by a spermatium nucleus containing factor S_2 it is found, judging from experimental results, that from the zygote S_1 S_2 from this cross a segregation within the ascus takes place distributing the factors so that four of the haploid nuclei contain compatibility factor S_1 and the remaining four contain compatibility factor S_2. The mycelium from haploid uninucleate spores, isolated from this cross, produce both male and female sex organs. It is inferred therefore that the spermatial nuclei contain the genetical qualities of bisexuality, and that the female nuclei in the ascogonia contain the genetical qualities of bisexuality. Thus even though the sex organs of each class are morphologically and functionally distinct at the reproductive phase of the life cycle, nevertheless the nuclei of each comprise the potentialities of both sexes.

SUMMARY

1. In *Pleurage anserina* the mycelia derived from uninucleate ascospores are hermaphroditic, self-sterile but capable of cross-fertilization.

2. All of the cultures from uninucleate ascospores fall into two classes, *A* and *B*, on the basis of compatibility; all cultures from single uninucleate spores belonging to class *A* are self-sterile and inter-sterile; all cultures from single uninucleate spores of class *B* are self-sterile and inter-sterile, but any one or all cultures derived from uninucleate spores of class *A* are reciprocally fertile with any one or all cultures derived from single uninucleate spores of class *B*.

3. The cultures derived from the normal binucleate ascospores appear to be homothallic, *i.e.* hermaphroditic and self-fertile, as cultures from single ascospores readily develop perithecia; however, a study based upon hyphal tips shows that the individual nuclei differ from each other, not in sex but in compatibility, each nucleus retaining its individuality so that mycelia from a hyphal tip containing nuclei of the same compatibility class will give rise to both male and female organs, but will prove self-sterile. The mycelia arising from a normal binucleate ascospore therefore, although appearing to be homothallic, that is hermaphroditic and self-fertile, in reality comprise two hermaphroditic cultures growing intermingled as one, each giving rise to both male and female organs which are self-sterile but which are reciprocally fertile.

4. It has previously been pointed out that cultures of class *A* and *B* are each hermaphroditic; but there is also evidence that within each nucleus contained in the male and female organs of each class are the genetical qualities of bisexuality.

5. If we represent the difference of compatibility between class *A* and *B* by two factors S_1 and S_2 respectively we may assume, from experimental evidence, that factor S_1 and S_2 are allelomorphic and that associated with each are sex factors.

6. The sexuality of the giant multinucleate ascospores is essentially like that described for the normal binucleate ascospores, except that there are more initial nuclei concerned; these nuclei belong to two classes, each of which gives rise to both male and female organs which are self-sterile but reciprocally fertile.

7. Because it has been found that cultures from uninucleate ascospores of *P. anserina* are bisexual self-sterile and cross-fertile, it is no longer justifiable to conclude that a culture from a single spore is unisexual simply because it does not produce fruiting bodies when grown alone, or because it requires the mating of complementary mycelia; also it is no longer justifiable to conclude that a culture derived from a single spore is simply bisexual and self-fertile because it does produce fruiting bodies when grown by itself.

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CYTOLOGICAL STUDIES IN THE TREMELLACEAE
I. TREMELLA

R. M. Whelden [1]

(with plates 47–49 and 11 text figures)

INTRODUCTION

Only one of the comparatively few cytological studies in the Basidiomycetes has been a comprehensive treatment of the Tremellaceae; and even this one, by Neuhoff (1924), (14), dealt mostly with the reproductive cells. Considering the position of the group, recognized as low in the Basidiomycete line, it would seem worthy of an intensive study. The present paper records a part of the observations resulting from a study as complete as possible of the members of the group found in New England.

HISTORICAL BACKGROUND

From the time when Tulasne (17), in 1853, commented on the absence of "spermatia" (= conidia) in certain species of *Tremella* to that when Brefeld (2), in 1888, concluded his long continued cultural studies in the Protobasidiomycetes, little was done other than taxonomically. Brefeld concluded that form, size, variations in color, etc., were of no classificatory value, but that the shape of the conidia, meaning secondary spores, or sporidia, was of prime importance; this enabled him to separate *Exidia,* with its sickle-shaped spores, from *Tremella,* with its spherical spores, but forced him to establish *Ulocolla* as a new genus to care for *Exidia saccharina* with its straight cylindrical conidia. Möller (13) even more emphatically asserted the uselessness of external appearances in describing Protobasidiomycetes, and stated that the shape of the hymenium, the form of the basidia, the sterigmata and the

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spores were all essential, as well as the details of spore germination.

The first real cytological study in the Tremellaceae is that of Istvánffi (8), in 1895, who followed the external changes in spore outline during development and also the formation of the basidium, which he considered uninucleate when young, through to the completion of sterigmata formation in *Tremella lutescens*. At about the same time, in 1895, Dangeard (4) followed accurately the movements and changes in shape of the nuclei from their fusion in the basidium through their migration into the spore, and in *Tremella mesenterica*, stated that the nucleolus became separated from its nucleus during its entrance into the spore. He also described the changes in shape of the fruit-body as its size increased. In 1902, Maire (11), in a comprehensive survey of the whole range of Basidiomycetes and with a very complete bibliography, described the chromosomes as being formed from the fusion of many small discrete chromatin granules which he called protochromosomes. He noted that in *T. mesenterica* the conidia which Dangeard (5) found to be ultimately uninucleate, were always binucleate as were the secondary conidia budded off from them.

Much more recently, in 1924, Neuhoff (14), after a careful comprehensive study of the Auriculariaceae and Tremellaceae, described the basidium as composed of two parts, the basal inflated part in which nuclear fusion and reduction divisions occurred being the hypobasidium from which two to four elongated apical outgrowths, called epibasidia, developed and which ended in slender tubes, the true sterigmata, an interpretation of the basidium accepted by most subsequent students of the Protobasidiomycetes. Neuhoff concentrated his studies mostly on the development of the basidium, but did go into other structures more intensively than had any previous workers. He had only moderate success in *Exidia*, being for the most part unable to follow the stages of nuclear activity. In the case of conidia, he found that "Erst durch die zytologischen Untersuchungen ist ein wesentlicher Unterschied aufgedeckt worden: die am einkernigen Mycel oder an den Sporen erzeugten Konidien sind einkernig (cf. *Exidia glandulosa, E. truncata,* and *E. saccharina*) die in d. Fruchtkörpern gebildeten Konidien besitzen zwei Kerne (*Tremella foliacea, T. lutescens*)." Neuhoff did not follow the development of clamp

connections, which he found to be common only in *Tremella indecorata*.

Shortly after Neuhoff's paper appeared, Kühner (9), in 1926, briefly noted the results of a study of *Tremella gemmata* Lév., *Sebacina gloeocystidiata* Kuh., and *Protohydnum lividum* Bres. In *Tremella* he doubtfully stated two chromosomes to be present in the basidium, while his rather poor figures indicate three: he gave no description of nuclear activity in this genus nor in *Sebacina*. In the latter genus he found centrosomes definitely present and also noted in the gloeocystidium the existence of spiral bands which he considered to be vestiges of cytoplasm.

From this brief enumeration of papers dealing with the cytology of the Tremellaceae, it would seem that an intensive cytological study of as many members of the family as possible would be in order and would do much towards furnishing a better basis for connecting this family with the other groups of fungi.

MATERIAL AND METHODS

That the results be as near normal as possible, the material used in the present study was gathered and fixed in the field during periods of heavy rain, a fact in itself desirable in that it rendered more noticeable fruit-bodies usually overlooked in a dry condition, and also eliminated to a great degree the presence of Fungi Imperfecti so commonly found in the "jelly" of the Tremellaceae.

In the early period of this study, two questions arose: was there any particular time when nuclear activity in the basidium was at a peak, and were there any particular changes to be noted when desiccation occurred? To answer these, several collections were carried on at hour intervals through a 24-hour interval both in rainy weather and during drying. It was discovered that the only factor affecting vital activity was the length of time of exposure to wetting. Drying produced very evident thick walled hyphae forming a protective layer over the surface of the fruit-bodies of *Exidia*, but almost no changes in the other genera studied.

After numerous preliminary fixings in Flemming's Weak Solution, in a picro-mercuric chlorid-alcohol solution,[2] in a chrom-acetic

[2] Picric acid, sat. sol. in 50 per cent alcohol .... 100 c.c.
Glacial acetic acid .... 7 c.c.
Mercuric chlorid .... .5 c.c.

spores were all essential, as well as the details of spore germination.

The first real cytological study in the Tremellaceae is that of Istvánffi (8), in 1895, who followed the external changes in spore outline during development and also the formation of the basidium, which he considered uninucleate when young, through to the completion of sterigmata formation in *Tremella lutescens*. At about the same time, in 1895, Dangeard (4) followed accurately the movements and changes in shape of the nuclei from their fusion in the basidium through their migration into the spore, and in *Tremella mesenterica*, stated that the nucleolus became separated from its nucleus during its entrance into the spore. He also described the changes in shape of the fruit-body as its size increased. In 1902, Maire (11), in a comprehensive survey of the whole range of Basidiomycetes and with a very complete bibliography, described the chromosomes as being formed from the fusion of many small discrete chromatin granules which he called protochromosomes. He noted that in *T. mesenterica* the conidia which Dangeard (5) found to be ultimately uninucleate, were always binucleate as were the secondary conidia budded off from them.

Much more recently, in 1924, Neuhoff (14), after a careful comprehensive study of the Auriculariaceae and Tremellaceae, described the basidium as composed of two parts, the basal inflated part in which nuclear fusion and reduction divisions occurred being the hypobasidium from which two to four elongated apical outgrowths, called epibasidia, developed and which ended in slender tubes, the true sterigmata, an interpretation of the basidium accepted by most subsequent students of the Protobasidiomycetes. Neuhoff concentrated his studies mostly on the development of the basidium, but did go into other structures more intensively than had any previous workers. He had only moderate success in *Exidia*, being for the most part unable to follow the stages of nuclear activity. In the case of conidia, he found that "Erst durch die zytologischen Untersuchungen ist ein wesentlicher Unterschied aufgedeckt worden: die am einkernigen Mycel oder an den Sporen erzeugten Konidien sind einkernig (cf. *Exidia glandulosa, E. truncata,* and *E. saccharina*) die in d. Fruchtkörpern gebildeten Konidien besitzen zwei Kerne (*Tremella foliacea, T. lutescens*)." Neuhoff did not follow the development of clamp

connections, which he found to be common only in *Tremella indecorata.*

Shortly after Neuhoff's paper appeared, Kühner (**9**), in 1926, briefly noted the results of a study of *Tremella gemmata* Lév., *Sebacina gloeocystidiata* Kuh., and *Protohydnum lividum* Bres. In *Tremella* he doubtfully stated two chromosomes to be present in the basidium, while his rather poor figures indicate three: he gave no description of nuclear activity in this genus nor in *Sebacina.* In the latter genus he found centrosomes definitely present and also noted in the gloeocystidium the existence of spiral bands which he considered to be vestiges of cytoplasm.

From this brief enumeration of papers dealing with the cytology of the Tremellaceae, it would seem that an intensive cytological study of as many members of the family as possible would be in order and would do much towards furnishing a better basis for connecting this family with the other groups of fungi.

MATERIAL AND METHODS

That the results be as near normal as possible, the material used in the present study was gathered and fixed in the field during periods of heavy rain, a fact in itself desirable in that it rendered more noticeable fruit-bodies usually overlooked in a dry condition, and also eliminated to a great degree the presence of Fungi Imperfecti so commonly found in the "jelly" of the Tremellaceae.

In the early period of this study, two questions arose: was there any particular time when nuclear activity in the basidium was at a peak, and were there any particular changes to be noted when desiccation occurred? To answer these, several collections were carried on at hour intervals through a 24-hour interval both in rainy weather and during drying. It was discovered that the only factor affecting vital activity was the length of time of exposure to wetting. Drying produced very evident thick walled hyphae forming a protective layer over the surface of the fruit-bodies of *Exidia,* but almost no changes in the other genera studied.

After numerous preliminary fixings in Flemming's Weak Solution, in a picro-mercuric chlorid-alcohol solution,[2] in a chrom-acetic

[2] Picric acid, sat. sol. in 50 per cent alcohol .... 100 c.c.
Glacial acetic acid .... 7 c.c.
Mercuric chlorid .... 5 c.c.

mixture,[3] in Bouin's solution, in Gilson's solution, in Navashin's solution, and others, the second and third solutions were used entirely. Parts of every collection were fixed in each of the chosen solutions.

Thoroughly washed fixed material was embedded part in celloidin and part in paraffin, after very slow dehydration through ten grades of alcohol. Celloidin sections gave greater ease in orienting material and permitted cutting substratum as well as fungus; paraffin sections were thinner. Except in details of the fusion nucleus, where sections were 4 μ, all sections were cut from 8–10 μ thick.

Haidenhain's iron-alum-haematoxylin gave the most satisfactory results, except in those cases where extranuclear material stained almost exactly like the nucleus. As contrast stains, erythrosin, fast green, methyl blue, and safranin gave approximately equally satisfactory results, if allowed to stain only lightly. Delafield's haematoxylin was also used. To overcome the difficulty mentioned above, other stain combinations such as Flemming's Triple, Mallory's Triple, etc., were tried, but with no particular advantage over Haidenhain's. Cajal-Brožek's method, as described by Hruby (7), did, however, prove excellent as a stain for dividing nuclei; it was found necessary to increase greatly the time for each of the stains used therein. No one stain proved universally good, but since all material was stained in three or more combinations after each of the two fixing fluids mentioned, reasonably satisfactory results were assured.

TREMELLA

Due to their general habit of growing singly or in small isolated groups, and, in New England, at least, seeming to reach maximum development in cool or even cold weather, members of the genus *Tremella* have not been collected as abundantly as have the other genera used in the present study. All species here studied were found on dead twigs and branches of various deciduous-leaved trees. Identification, especially of young material, was often a matter of considerable difficulty and resulted in the rejection of

[3] Chromic acid	7	gr.
Glacial acetic acid	7.5	c.c.
Distilled water	1000	c.c.

several collections in which the identity was doubtful. All identifications are based on the descriptions of Bourdot and Galzin (1), supplemented by references to many other descriptions. The species studied are *Tremella mesenterica* Retz., found abundantly (several collections of *Tremella lutescens* Pers., here considered identical with *T. mesenterica,* since in all collections determined as such, cytological details were identical in every particular, so bearing out the statements of Neuhoff (15), Miss Looney (10), and others who doubt their separate identity), *Tremella frondosa* Fries,[4] and *Tremella Grilletii* Boud. (*Tremella glacialis* Bourd. & Galz. here is considered identical with *T. Grilletii.*)

Habit

The youngest fruit-bodies of *Tremella mesenterica* Retz. seen had a uniformly smooth surface which became irregularly lobed and folded with increase in size. Except for the pale translucent grey color of the very youngest bodies, all were of some shade of yellow, commonly near Baryta Yellow[5]; the consistency was a firm gelatinous to a soft almost slimy condition when thoroughly wetted. Young fructifications of *T. frondosa* were Light Seal-Brown in color and made up of a single rather firm lobe; old fruit-

[4] The specific identity of this material has been a problem. In color, size, and shape of fruit-body, all recognized as of doubtful value in determining species of *Tremella,* these would be considered *T. frondosa.* Measurements of various parts are equally doubtful, as the following table shows:

Authority	Basidia	Spore	Epibasidia	Hyphae	Conidia
The writer	$8 \times 6\,\mu$	$5.2 \times 3.5\,\mu$	$21 \times 1\text{–}2\,\mu$	$1.2\text{–}2.5\,\mu$	$2.5 \times 2\,\mu$
Bourdot & Galz. (1)	$14\text{–}18\text{–}24 \times 11\text{–}12\text{–}18\,\mu$	$7.5\text{–}10 \times 7\text{–}9\,\mu$	$30\text{–}45 \times 2\text{–}6\,\mu$	$1\text{–}2\text{–}6\,\mu$	$3\text{–}4.5 \times 2\text{–}3\,\mu$
Neuhoff (15)	$16\text{–}20(\text{–}24) \times 12\text{–}18\,\mu$	$7\text{–}10 \times 7\text{–}9\,\mu$			$3\text{–}4.5 \times 2.5\text{–}3\,\mu$
Martin & Huber (12)	$7\frac{1}{4}\text{–}14.8 \times 8\text{–}16\,\mu$	$7\text{–}9\,\mu$ diam.			
Saccardo (16)	$15\,\mu$ diam.	$5 \times 7\,\mu$			

Several workers, as Bourdot and Galzin (1), Coker (3), and Miss Looney (10) have doubted the separate identity of *T. frondosa.* Others, as Gilbert (6), consider it as a good species; the writer's specimens resemble very closely Gilbert's photographs and description. In view of the lack of agreement of descriptions it is to be questioned whether there may not be two species confused under the name *T. frondosa.* Certainly the specimens studied here are distinct from all others studied.

[5] All capitalized colors are taken from Ridgeway's Color Standards and Nomenclature.

bodies varied from Light Seal-Brown to Dilute Cinnamon-Brown, according to moisture conditions during growth, and were very large, the whole lobed body being 30 cm. long and 7 cm. in diameter across lobes. The changes in *Tremella Grilletii* Boud. are of greater interest. The youngest fruit-bodies studied, collected on rotten Elm in September, are discrete bodies from 25 μ to 0.6 mm. in diameter, nearly colorless and with many mature spores, 8.5 $\times$ 6 μ, present on the upper surface. As time went on, the fruit-bodies expanded over the surface of the substratum until in November, some tended to become confluent, although showing their individuality when sectioned. From mid-January to May, however, confluence was complete, it being possible to peel off masses 2 sq. cm. in extent, sections of which showed absolutely no separation of units except at the very edge of the fruit-body. Because of this change and with a range of spore sizes from 8.5–6.5 $\times$ 6–3.5 μ, the writer is inclined to doubt the validity of separating *T. glacialis* Bourd. & Galz. from *T. Grilletii* Boud.

Internal Structure

The mycelium of the species of *Tremella* studied may be traced well back into the substratum, where it is composed of monotonously uniform binucleated segments from 1–1.8 μ in diameter. (PLATE 47, FIG. 1, A, C; PLATE 48, FIG. 1, D-E.) On emergence to form a fruit-body the hyphae differ somewhat from this mycelium, generally becoming slightly larger, more twisting and exhibiting certain specific differences. In *T. mesenterica,* for example, clamps are uncommon and the nuclei of any one segment quite remote from one another (PLATE 47, FIG. 1, B); in *T. frondosa* clamps are very numerous and the nuclei usually close together near the middle of a segment (PLATE 48, FIG. 1, A, B). In these two species considerable quantities of a densely staining substance accumulate at the ends of the segments or even in the middle. No great quantity of this substance was observed in *T. Grilletii,* where other differences were also noted. In this minute species, the hyphae, on emergence from the substratum either spread out in a radial manner to form a minute fruit-body or formed a much tangled thin mat of short binucleate segments over the surface of the wood (PLATE 49, FIG. 1). No clamp connections were seen in this species.

Hypobasidium

As the fruit-body develops it is noticed that there is a dense accumulation of protoplasm in the hyphal tips which form the surface layer. In these dense tips, the basidium initials, the two small nuceli, exactly like those of the mycelium in size and shape, move together and fuse (PLATE 47, FIG. 2; PLATE 48, FIG. 2; PLATE 49, FIG. 2, 3). Even before this fusion occurs, the hypobasidium starts swelling to a spherical to somewhat fusiform body while the hyphal stalk which it ends becomes more and more vacuolate up to the place at which the basal septum will presently form.

With fusion of the two nuclei come several very definite changes: the minute inconspicuous nucleoli fuse to form a very evident large nucleolus (PLATE 48, FIG. 3); the apparently structureless condition of the primary basidial nuclei is replaced by a very noticeable structure which becomes increasingly evident with increase in size, which is very great, both in the hypobasidium and in the included fusion nucleus, particularly in *T. mesenterica* and *T. Grilletii* (PLATE 47, FIG. 3; PLATE 48, FIG. 3–6; PLATE 49, FIG. 4–9). Increase in size produces certain very characteristic specific differences; in *T. mesenterica,* the fusion nucleus, 5 μ in diameter, occupies the center of the very large sub-spherical to irregular hypobasidium whose dimensions average $15 \times 9.7\ \mu$ (PLATE 47, FIG. 4); in *T. frondosa* the maximum diameter of the fusion nucleus is 2.8 μ while that of the very irregularly shaped hypobasidium averages $8 \times 6\ \mu$ (PLATE 48, FIG. 6, 8); in *T. Grilletii* the fusion nuclei in any single fruit-body are very uniform in rate of development to attain a maximum diameter of 3–3.5 μ in the nearly spherical hypobasidia 5–6 μ in diameter (PLATE 49, FIG. 10, 11). Almost from its first formation the chromatin of the fusion nucleus shows itself organized into what at first seems to be an irregular reticulum, but which as development continues becomes more evidently organized into linear patches scattered about at the surface of the nucleus. With continued increase in size these become definitely eight masses of chromatin which gradually contract to form the eight small spherical to elliptical chromosomes (PLATE 47, FIG. 5, A; PLATE 48, FIG. 9; PLATE 49, FIG. 4, 12–14). Thes prochromosomes have been found in every species of the tremellaceous fungi so far studied. With the contraction

of the chromosomes the nucleolus gradually becomes less distinct, finally disappearing at about the time when the chromosomes are definitely formed; the nucleolus seems always to be located at the surface of the nucleus, but never extruded therefrom. As the eight chromosomes separate in reduction division the nuclear membrane disappears, leaving the two groups of four often closely clumped chromosomes to migrate to opposite sides of the hypobasidium. (PLATE 47, FIG. 5, B; PLATE 48, FIG. 7; PLATE 49, FIG. 14.) No definite spindle has been observed in any nuclear division in *Tremella*. The daughter nuclei, much larger in *T. mesenterica* than in the other two species, become definitely reorganized before the second nuclear division occurs (PLATE 47, FIG. 6, 7;

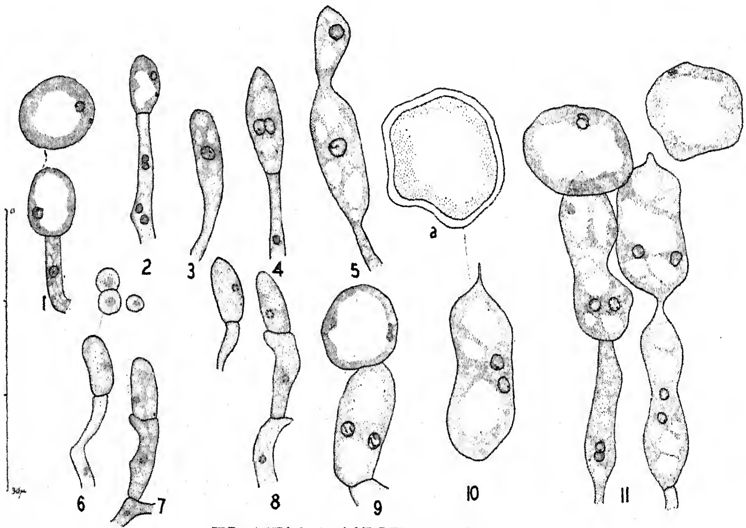

FIG. 1, Uninucleate spherical abortive body; 2, 3, 4, 5, Transitional stages between the uninucleate bodies such as shown in fig. 1 and the binucleate bodies shown in fig. 9; 6, Conidia-like cells embedded in "jelly," with tip of hyphae from which they form; 7, 8, Chains of uninucleate non-spore-like bodies; 9, Large binucleate abnormal "conidia"; 10, 11, Stages in development of abnormal binucleate bodies.

PLATE 48, FIG. 11, 12; PLATE 49, FIG. 15). This second division, with the four chromosomes splitting in halves, occurs either simultaneously (PLATE 48, FIG. 15; PLATE 49, FIG. 16, 18) or one nu-

cleus divides completely before the other starts to divide (PLATE 48, FIG. 14; PLATE 49, FIG. 22). Often the second division fails to take place, particularly in *T. Grilletii*. The four nuclei usually lie at the same level about midway in the hypobasidium (PLATE 47, FIG. 8; PLATE 48, FIG. 13; PLATE 49, FIG. 21), and show a characteristic structure, having a definite small nucleolus and a loosely arranged superficial network of chromatin seeming always to be separable into four groups at all times; this is most easily seen in the relatively large nuclei of *T. mesenterica* (PLATE 47. FIG. 8).

Septum formation has no direct connection with the divisions of the nucleus, indeed, the time of formation of the various septa is extremely variable, but seems rarely to occur until the first division is completed; then the basal and first longitudinal septa rapidly form, the latter apparently basipetally (PLATE 47, FIG. 7; PLATE 48, FIG. 14–16; PLATE 49, FIG. 15–18). The second longitudinal septum is formed immediately after the second division. The planes of formation are extremely variable, although usually nearly central in the hypobasidium.

Epibasidia

Even before the second nuclear division occurs the two or four (rarely three) epibasidia appear as hemispherical bulges filled with a dense accumulation of protoplasm on the apical surface of the hypobasidium. With increasing elongation there appear certain interesting variations which necessitate individual treatment of the three species. In *T. mesenterica* the epibasidia rapidly develop to coarse, slightly twisting structures from 2–3.5 μ in diameter, projecting more or less directly upward from the hypobasidium to the surface of the fruit-body above which they may protrude to a distance of 15 μ, frequently becoming very slightly enlarged (PLATE 47, FIG. 6–12). With development of the epibasidia there occurs a vacuolation of the hypobasidium which progresses from the base of the latter until even the basal portion of the mature epibasidium is included. The development of the epibasidia in *T. frondosa* is even more interesting; here the very slender structures, from 0.8–2 μ in diameter, show definite extremes of development. In the first extreme, which seems to occur mostly at the top of the lobes of the fruit-body, the nearly

straight objects gradually increase in diameter as they push out to the surface of the "jelly" and beyond, and terminate abruptly in the slender sterigmata (PLATE 48, FIG. 17–19). In the second extreme, found mostly at the base of the lobes, the epibasidia continue outward with practically uniform diameter until well beyond the surface of the "jelly" and then expand abruptly to a pronounced one-sided head often $4.8 \times 8\ \mu$, from which the very slender sterigma generally develops laterally (PLATE 48, FIG. 20, 21 C). In *T. Grilletii,* epibasidial development is more uniform: in the younger fruit-bodies mentioned earlier the usually four, but often two, epibasidia are rather chunky objects which just reach to the surface of the "jelly" (PLATE 49, FIG. 19, 20); in the older confluent fruit-bodies, they are longer, more slender structures, generally four to each hypobasidium (PLATE 49, FIG. 22, 24, 25).

As elongation of the epibasidia occurs nuclear migration begins, its first indication being the definite elongation of the nuclei, an elongation which becomes more pronounced as they approach the bases of the epibasidia (PLATE 47, FIG. 10–12). The rate of movement into and through the epibasidia appears to be rather unequal, so that the various nuclei are at different regions in the respective epibasidia (PLATE 47, FIG. 12; PLATE 49, FIG. 25). Subsequent to the passage of the nucleus the protoplasm becomes decidedly vacuolate, the hypobasidium becoming almost empty. As the nucleus passes along the epibasidium there develops from its apical end a slender tube, the sterigma. To pass through this, the nucleus necessarily becomes extremely elongated, the elongation frequently occurring before the nucleus reaches the sterigma (PLATE 48, FIG. 21, C, D).

Spores

Except for the differences in size, the spores of the three species of *Tremella* studied show remarkable similarity in manner of development and germination. The earliest appearance of the spore is a small spherical bubble at the end of the sterigma (PLATE 47, FIG. 12; PLATE 48, FIG. 21, D). This bubble rapidly enlarges to a spherical object with a pronounced apiculus lateral to its attachment to the sterigma (PLATE 47, FIG. 20; PLATE 48, FIG. 21 B, 22; PLATE 49, FIG. 23, 26). When mature the spores of *T. mesen-*

terica average $9 \times 7\ \mu$ and have a pronounced dense apical protoplasmic cap (PLATE 47, FIG. 21); those of *T. frondosa* average $5.2 \times 3.5\ \mu$ (PLATE 48, FIG. 23); while those of *T. Grilletii* are $6 \times 3\ \mu$; all have vacuolate protoplasm, but particularly *T. Grilletii* (PLATE 49, FIG. 27, 28).

On germination, the germ tube (very rarely two form) protrudes, usually laterally (PLATE 47, FIG. 22, 23; PLATE 48, FIG. 24; PLATE 49, FIG. 29–37); at the same time the nucleus starts migrating towards its base, becoming elongated in that direction in doing so (PLATE 47, FIG. 24). Passage through the germ tube into the secondary spore shows exactly the same phenomena as were shown during nuclear migration through the epibasidium, with the primary spore wall collapsing when the nucleus enters the secondary spore (PLATE 47, FIG. 25, 26). This secondary spore is very like the primary spore except in its smaller size (PLATE 47, FIG. 27; PLATE 49, FIG. 36). During germination, no nuclear division occurs. Tertiary spores are apparently also formed at times.

Conidia

Two types of conidia occur in *Tremella*. One of these was seen only in *T. frondosa* and in this species only when spores were germinated in water. From these spores there developed from one to four chains of two to three cells each, budded off from the primary spores. These conidia were always uninucleate; no nuclear divisions were seen in their formation (PLATE 48, FIG. 27).

In *T. mesenterica,* a second type of conidium developed from hyphal tips located among and around groups of hypobasidia. These hyphal tips become many times short septate with each segment usually binucleate. Gradually these segments round off to form spherical binucleate conidia 2.5 to $3\ \mu$ in diameter (PLATE 47, FIG. 16). In many cases the two small nuclei lie one above the other in the plane of vision and are difficult to distinguish; in other cases, particularly in older conidia, the two nuclei seem to fuse, although this blending may be due to approaching disintegration of the contents rather than normal development. Probably the small bodies formed apically on the hyphae of *T. frondosa* (PLATE 48, FIG. 26) are to be interpreted as conidia; these, however, were always found to be uninucleate. Also interpreted as conidia are

the small cylindrical bodies, $5 \times 1.5\ \mu$, found at the ends of slender collapsed tubes in fruit-bodies of *T. Grilletii;* these were binucleate bodies with uniform content of densely staining protoplasm (PLATE 49, FIG. 39).

Abortive structures

Frequently in the fruit-bodies of different species of *Tremella* are found structures perhaps best regarded as abortive, which have been used at various times to explain rather unconvincingly a connection between basidia and conidia, but in any case, of considerable interest. It is difficult to divide these into separate groups on any other basis than that of nuclear condition, some being uninucleate, others binucleate. Through disintegration of the nuclei during development, binucleate bodies become or appear uninucleate; no nuclear fusions ever seem to occur (PLATE 47, FIG. 19; PLATE 48, FIG. 30).

In the binucleate structures the first condition seems always to be a short segmented hypha, in each segment of which are two distinct nuclei (PLATE 47, FIG. 13; TEXT FIG. 2, 4). As development proceeds, the ultimate segment rapidly enlarges, and becomes more and more vacuolate (PLATE 47, FIG. 14; PLATE 48, FIG. 28), until eventually it is a somewhat spherical body having a thin peripheral layer of protoplasm in which are seen the two adjacent disintegrating nuclei (PLATE 47, FIG. 15; PLATE 48, FIG. 29). Eventually this structure becomes a hollow slightly wrinkled empty cell with a conspicuously thick wall, and 10–16 μ in diameter (PLATE 47, FIG. 18). Frequently it becomes separated from the basal portion, often showing a pronounced projection where it was attached (PLATE 47, FIG. 17). Rarely a second spherical cell like the first is formed below it, but more frequently it fails to form at all, or becomes an elongate, curved cylindric, binucleate cell containing a highly vacuolate protoplasm (TEXT FIG. 9, 10). This cell may become a large spindle-shaped cell ($15–20 \times 5–8\ \mu$), which usually ends in a pronounced prong distally and is attached to a similar but more slender segment below (TEXT FIG. 11).

It might be easy to interpret the uninucleate bodies as formed by segmentation of the last described binucleated condition were it not that from their earliest appearance, each segment is obviously

uninucelate. These uninucleate structures apparently fall into two distinct groups; one, seen only in *T. mesenterica* (TEXT FIG. 6, 7, 8) being a short series of segments, of small diameter, from which there seem to form uninucleate conidia-like bodies which at first have very thin uniform protoplasm and eventually become small empty spherical shells embedded in the "jelly." The second uninucleate structure occurs as a chain of large cells which rapidly swell to form spherical to short cylindric segments from 4–8 μ in diameter and often occurring in large much crowded branching groups (TEXT. FIG. 3, 5; PLATE 48, FIG. 33, 36).

It is interesting to note that abortive structures are conspicuously few in *T. Grilletii* (see, however, PLATE 49, FIG. 38), where one encounters but rarely hypobasidia-like structures in which the two small nuclei remain close together without fusing in the highly vacuolate protoplasm (PLATE 49, FIG. 40). Similar structures occur in *T. frondosa* (PLATE 49, FIG. 10, 32). These hypobasidia-like bodies become more and more vacuolate until eventually they collapse and are lost in the developing fruit-body, a fate which is shared by all these structures described as abortive.

DISCUSSION

It is proposed to leave discussion of the possible significance of the various bodies above described until the completion of the present studies. There are, however, certain points which can best be considered at present.

One of these points, namely, the difficulty of separating *Tremella* from *Exidia* has been noted by Brefeld, by Neuhoff, and by others. Brefeld (2) held that the form of the conidia (*i.e.,* secondary spores), spherical to "pip"-shaped in *Tremella* and sickle-shaped in *Exidia,* was sufficient for generic distinction. Dangeard decided that the mere presence of true conidia in *Tremella* distinguished this genus from *Exidia.* Neuhoff (15) accepted Fries's original separation based on the hymenium, which is unilateral in *Exidia* and amphigenous in *Tremella,* and also used Brefeld's criterion of spore shape in separating the two genera, but pointed out that their exact separation must rest on further research. In his 1931 paper (15), he stated that *T. Grilletii* Boud., and *T.*

glacialis Bour. & Galz. are really species of *Exidia* having the sickle-shaped spores which characterize the latter genus. With this statement the writer cannot agree, for all spores seen in the *Tremella Grilletii* here studied are of the typical *Tremella* shape.

Indeed, the writer does not consider that the shape of the primary spores or of the secondary spores developing from them offers the only means for separating the two genera. In the various species of *Tremella* and *Exidia* found in this region, there are two additional points of difference. The first of these is the presence in *Tremella* of the numerous "abortive" structures which are completely lacking in every species of *Exidia* studied. It must be admitted, however, that these bodies are lacking in *Tremella Grilletii,* unless FIG. 40 of PLATE 48 is to be interpreted as such. The second point of difference between the two genera appears when desiccation sets in. In *Tremella* there is then merely a general shrinking together of the parts of the fruit-body with little if any change in any single part. In Exidia, on the contrary, the superficial hyphae of the drying fruit-body become definitely modified to form a dense layer of thick-walled more or less interlacing hyphae which will be described in a future paper.

The second point which should be considered is the noticeably scanty cytological studies of the genus *Tremella.* Istvanffi's study (8) of *T. lutescens* and *T. Genistae* needs little comment; he followed mostly the development of the basidium as a whole, noting the formation of the septa whose development he found so easy to follow, but recording little concerning nuclear phenomena save that one nucleus was present in the young basidium and that the spores were uninucleate. Dangeard's more exact study (4) of *Tremella mesenterica* Retz was also more a consideration of changes in appearance of the fruit-body, correlated with the formation of conidia followed by basidia, than it was a study of internal development. He did, however, correctly describe the early formation of the fusion nucleus and also the changes occurring when the nucleus passed into the spore.

Considering the relative ease with which specimens may be obtained, it is a surprising thing that, following Dangeard's work, very little attention was paid the genus *Tremella* until Neuhoff's

study. Even though Neuhoff's results are based on a study of insufficient material, they are surprisingly complete.

Nevertheless the work of Neuhoff does leave many gaps which the writer believes his own observations more completely fill because they are based on a large series of specimens, collected throughout the year and particularly in cool to cold weather when they are abundant, and yielded several hundred slides. In the case of the mycelial segments, the writer agrees with Dangeard, Neuhoff, and others that a binucleate condition usually obtains. In these segments, the writer finds that certain evident specific differences appear, as already noted; particularly the relative positions of the two nuclei, which are near together in the center of the segment in *T. frondosa* and *T. Grilletii,* and quite remote from one another in *T. mesenterica.* The writer finds also that clamp connections occur in *Tremella* much more frequently than Neuhoff reported, occurring regularly in both *T. mesenterica* and *T. frondosa.* Moreover, in the hyphal segments of *Tremella,* the presence of a deeply staining substance locally aggregated should also be noted, since this is another means of separating *Tremella* from *Exidia,* where such aggregations are not usually seen.

The development of the hypobasidium (the basidium of the earlier writers) is remarkably constant in all species of *Tremella* and also the related genera *Exidia* and *Sebacina.* Because of this uniformity few points of specific separation other than the size of the mature basidium and its included nuclei occur. The development of the hypobasidium heretofore has not been followed throughout. In the main the various previous studies have resulted only in a series of somewhat unconnected conditions which, for the most part, the writer's studies bear out. That fusion of two small primary basidial nuclei occurs is readily seen; that there is an immediate and considerable increase in the size of the fusion nucleus has also been observed although not specifically noted. In the literature there is but one reference to the details of the fusion nucleus, namely, Dangeard's description of the formation of chromosomes through the fusion of many small bodies, the protochromosomes. The writer is in complete disagreement with this conception. From its first formation the chromatin material of the fusion nucleus is aggregated in masses which become more

and more noticeably organized into definite linear structures, the prochromosomes, whose contraction gradually leads to the formation of the chromosomes. The number of chromosomes is always eight in the diploid condition or four in the haploid.

In the work of previous investigators, studies of the development of the epibasidia (sterigmata of earlier workers) are as scanty as are those of the basidia, and are for the most part directed towards the evaluation of the dimensions of the mature structures and a description of the migration of the nucleus into the spore. That a connection should exist between the nuclei of the hypobasidium and the formation of epibasidia has been argued in other groups of fungi. The present studies definitely demonstrate that such correlation does not exist, showing repeatedly the occurrence of hypobasidia containing two nuclei but having four epibasidia in well advanced stages of development. In other instances an hypobasidium may contain four nuclei but show no sign of formation of epibasidia. The dimensions of the latter seem to depend directly upon the amount of "jelly" present; since "jelly" formation depends on the amount of water absorbed by the fruit-body, obviously atmospheric conditions must have considerable effect on the dimensions of the epibasidia.

Even less variation occurs in the spores which have very definite dimensions in each species here studied. It seems worthy of note, however, that on spore germination no division of the nucleus occurs in species of *Tremella,* the large nucleus migrating into the secondary spore; in *Exidia,* on the contrary, germination of the spore is accompanied by nuclear division, so that many very small nuclei are formed. In the nuclear migration into the secondary spore, as well as in the migration of the nucleus through the epibasidium, the present studies have yielded no evidence that the nucleolus ever becomes separated from its nucleus, nor is there any indication that it is located in any particular region, such as the front of the migrating nucleus. Nor does the extremely elongated shape finally assumed by the nucleus seem imposed upon it merely because of the very narrow diameter of the sterigma; this attenuate condition obtains before the sterigma is reached.

Since so much emphasis has been placed on conidia as a means of separating *Tremella* from *Exidia,* it seemed of value to pay

particular attention to these bodies. The present studies indicate that the succession of basidial formation to conidial postulated by Dangeard does not take place, but rather that the conidia occur in scattered patches among the basidia. The conidia, borne on the hypae of the fruit-body are always binucleate, as Neuhoff has stated. There is evidence, as previously pointed out, that with age there may occur an apparent blending of the nuclei leading to a condition seemingly uninucleate. The conflicting statements regarding nuclear conditions in the conidia, which Dangeard held to be uninucleate and Neuhoff binucleate, may be explained by the uninucleate conidia-like bodies which the writer has described (TEXT FIG. 6). In the main the conidia seem to become engulfed in the increasing mass of "jelly" within which they finally disappear.

On the basis of present study, the writer is convinced that little of value either morphologically or cytologically may be expected from a study of a small number of fruit-bodies, but rather that a large number of specimens carefully collected and prepared is essential in studying the minute structures of the genus. While it is to be hoped that the present study is a step in the right direction and has filled in many of the existing gaps, yet obviously much more long continued patient study of many species will be necessary before adequate basis for taxonomic and phylogenetic relations will be available.

SUMMARY

Fruit-bodies of *Tremella mesenterica* Retz, *T. frondosa* Fries, and *T. Grilletii* Boud. are composed of binucleated segments. The two nuclei are far apart in *T. mesenterica* and close together in *T. frondosa* and *T. Grilletii*. The hymenium, covering the entire exposed surface of the fruit body, is composed of hypobasidia in which two nuclei fuse. This fusion nucleus shows from its inception a definite organization, its chromatin material being aggregated in linear patches, the prochromosomes. These gradually contract when the fusion nucleus reaches its maximum size, to form the eight small chromosomes found in every species studied. These chromosomes separate in reduction division and reorganize into two small nuclei which may divide simultaneously

or individually. Subsequently, these small nuclei become somewhat elongated as they migrate into the epibasidia extending apically from the hypobasidium out to the surface of the "jelly" or beyond. When the nucleus reaches the end of the epibasidium its elongation becomes quite pronounced. Almost no variation in this process occurs in the different species, nor do the spores differ, except in size. These spores, uninucleate bodies borne at the tip of the sterigmata, germinate by a lateral germ-tube and form a secondary spore quite like the primary spore; in this process no nuclear division occurs.

Exceptionally spores give rise to short chains of uninucleate conidia. Conidia are also formed on hyphal tips in and around the basidia, and are binucleate.

Many other bodies also occur in this region. Some of these are uninucleate and in part resemble conidia; others binucleate. The presence of these "abortive" structures in *Tremella* seems to separate this genus from *Exidia,* which lacks them.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to Prof. W. H. Weston, Jr., for his continued encouragement and helpful criticism throughout the preparation of the present paper.

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EXPLANATION OF PLATES

PLATE 47

Tremella mesenterica Retz.

In this and all other plates, as well as in text figures, all figures have been drawn with the aid of a Camera lucida, at a magnification of 3800 ×, and subsequently reduced to about 0.3, *i.e.*, to a magnification of about 1150 ×. In addition an absolute scale of dimension is included in all plates.

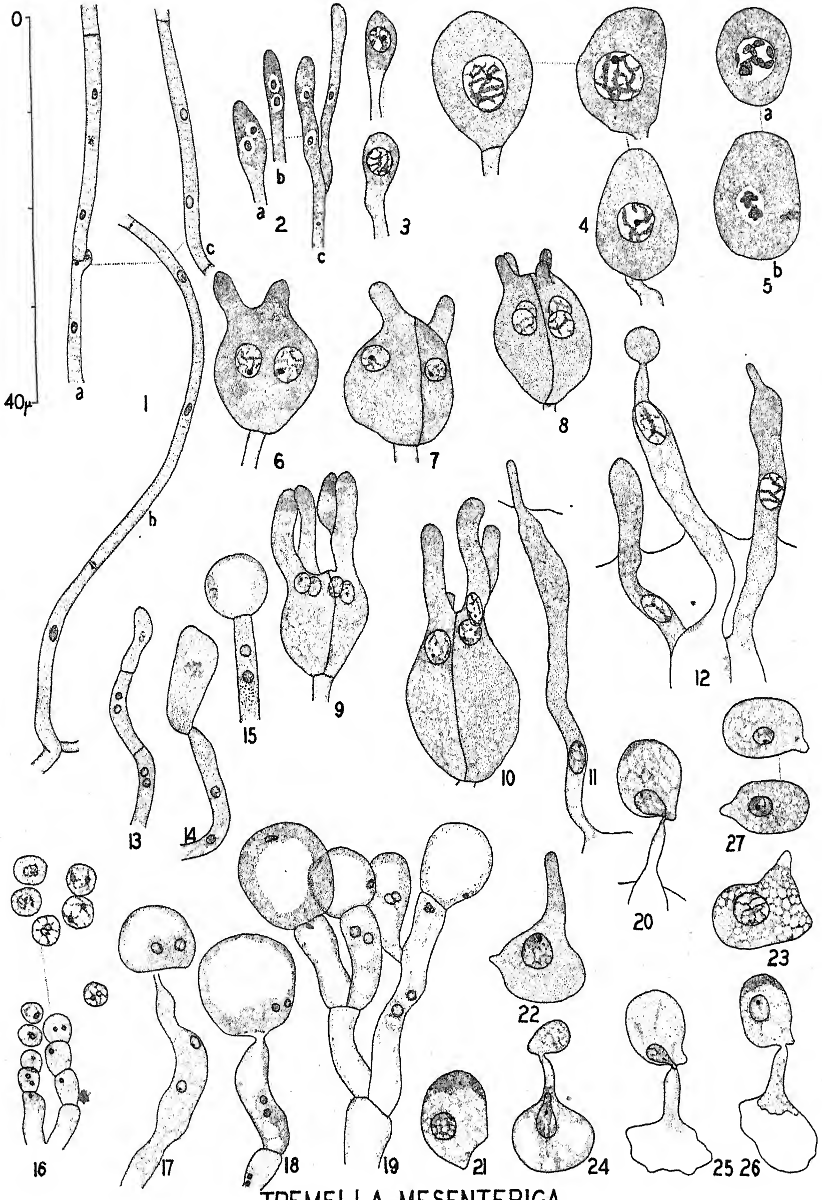
Fig. 1, *a, b, c*. Binucleate segments of hyphae, with oil-like substance accumulated near end-walls and also in the center of the segment in fig. *a*; 2, hybosadium initials, each showing the two nuclei before fusion; 3, hypobasidium showing the enlarging fusion nucleus and the forming basal septum; 4, hypobasidium showing the large mature fusion nucleus and the complete basal septum; 5, *a*, hypobasidium showing the nucleus in which are eight nearly mature chromosomes, *b*, the same with the chromosomes separating, four in each group; 6, hypobasidium showing the two daughter nuclei resulting from the first division and the two relatively thick epibasidia forming apically; 7, the same with the two daughter nuclei separated by the first longitudinal septum; 8, hypobasidium showing four basidial nuclei and four apical epibasidia; 9, hypobasidium showing the four nuclei migrating toward the bases of the half-developed epibasidia; 10, the same showing the slightly elongated nuclei entering the bases of the epibasidia; 11, epibasidium showing the elongated nucleus migrating toward the slender sterigma which projects above the surface of the "jelly"; 12, epibasidia showing the vacuolate protoplasm following the passage of the elongated nuclei. Sterigma of the central epibasidium bearing a half-formed spore; 13, binucleate segments of tip of hypha; 14, 15, stages showing the enlargement of the apical segment of a hyphal tip such as fig. 13; 16, binucleate conidia breaking loose from tip of chain. Upper two conidia to left showing vague blending of nuclei suggestive of fusion; 17, 18, chains of segments similar to those in fig. 14, but showing the penultimate segment becoming elongated and vacuolate; 19, branching group of binucleate cells similar to those

show in figs. 17, 18; 20, nearly mature spore showing the nucleus just leaving the sterigma and the apical "cap" of protoplasm beginning to form; 21, mature spore showing dense apical "cap" and somewhat vacuolate protoplasm; 22, 23, spore germinating by lateral germ tube; 24, germinating spore showing the elongating nucleus leaving the extremely vacuolate protoplasm of the spore to enter the germ tube at the tip of which a secondary spore is forming; 25, secondary spore showing the nucleus just entering; 26, nearly mature secondary spore at the tip of the germ tube; 27, mature secondary spores.

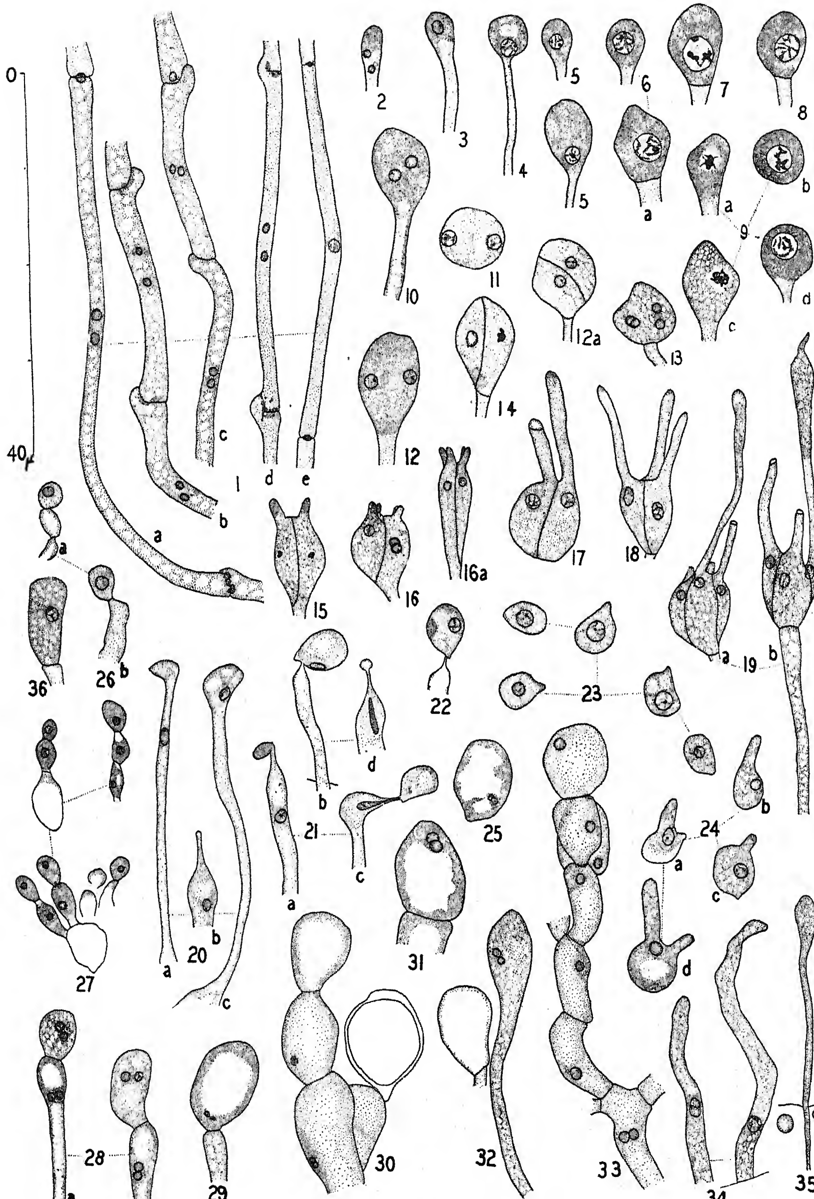
PLATE 48

Tremella frondosa Fries.

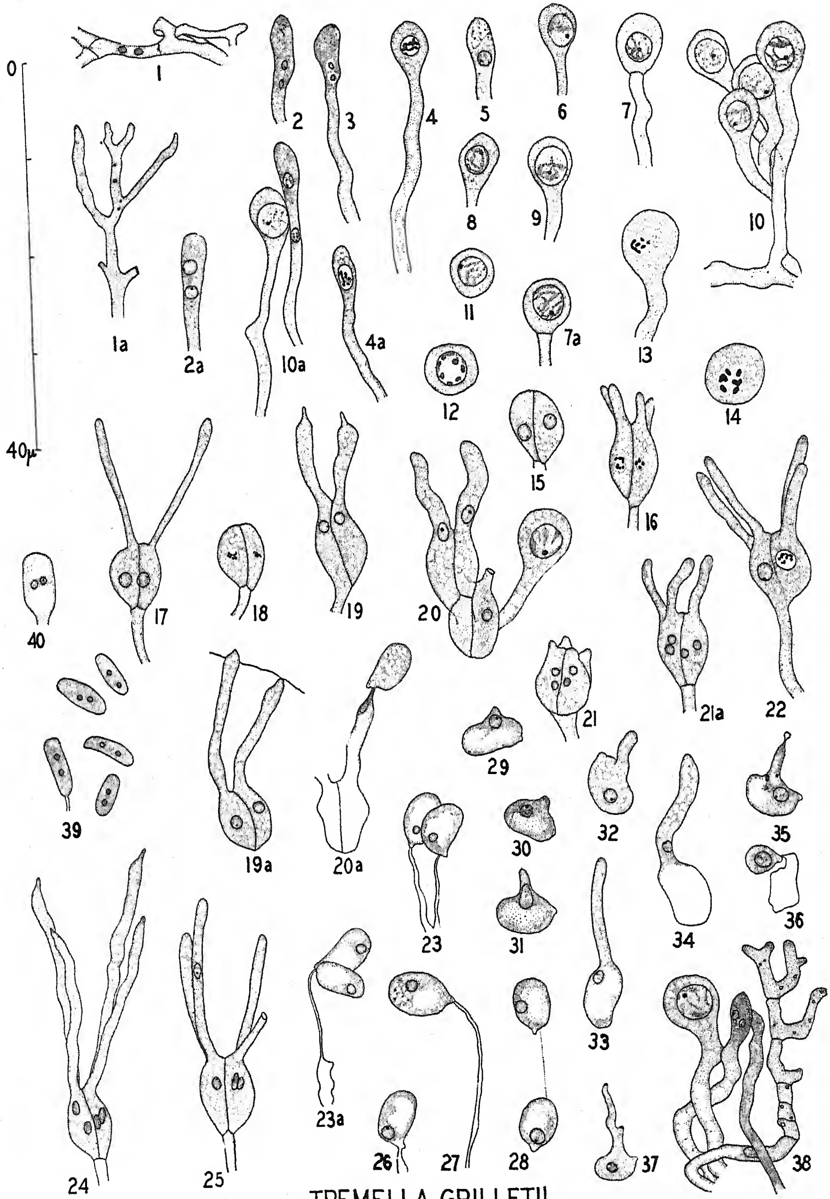
Fig. 1, hyphal segments: *a, b,* binucleate type found in fruit-body; *c,* the same beneath the hymenium showing the various arrangements of the two nuclei; *d,* binucleate segments in substratum; and e, uninucleate segment in substratum; 2, young hypobasidium with primary basidial nuclei before fusion; 3, same, showing the fusion nucleus before the nucleoli fuse; 4–6, hypobasidia showing enlarging fusion nucleus; 7, hypobasidium showing eight chromosomes in two equal groups; 8, same, showing mature fusion nucleus and the basal septum; 9, same, showing: *a, c,* the closely clumped chromosomes, and *b, d,* the diakinesis of the first division; 10, basidium-like binucleate hyphal tip; 11, cross section of hypobasidium showing two nuclei near the walls; 12, the same in side view; 12*a,* the same showing an oblique longitudinal septum separating the two nuclei; 13, hypobasidium showing four distinct nuclei; 14, same, showing one of the two nuclei dividing; 15, same, showing both nuclei dividing simultaneously and epibasidia forming apically; 16, hypobasidium showing four epibasidia forming at its apex. Basal septum as yet unformed; 16*a*, unusually elongate hypobasidium; 17, hypobasidium showing two well-developed apical epibasidia; 18, same, showing three well-developed apical epibasidia; 19, same, showing four epibasidia to the bases of which the nuceli are migrating; 20, epibasidia showing the nuclei near their swollen apices; 21, same, ending in slender sterigma directed either vertically (*d*) or laterally (*c*), at the tip of which the spore is forming. Nucleus either in the epibasidium (*a*) or very much elongated in the swollen epibasidial tip prior to passage through the slender sterigma (*c, d*), or in the spore close to the wall (*b*); 22, epibasidial apex showing the nearly mature spore at its tip; 23, mature uninucleate spores; 24, spores germinating by single lateral (*a, c*) or terminal (*b*) germ-tube, or (*d*) with two germ tubes; 25, spore-like body cut off from hyphal tip similar to that of fig. 10; 26, 27, uninucleate conidia cut off from hyphal tips, (26) or budding from spore (27); 28, binucleate cells in short chains at surface of fruit-body; 29, 31, stages in the formation of the spore-like body shown in fig. 25; 30, swollen vacuolate cells formed in chains and showing progressive nuclear disintegration. Thick-walled empty cell at right; 32, basidium-like hyphal tip with one of surrounding vacuolate segments, such as fig. 28; 33, branching chain of uninucleate segments generally found around the hymenium; 34, 35, erect hyphal tips which project rigidly above the surface of the fruit-body; 36, truncate uninucleate body sometimes occurring at edge of hymenium.



TREMELLA MESENTERICA



TREMELLA FRONDOSA



TREMELLA GRILLETII

PLATE 49

Tremella Grilletii Boud.

Fig. 1, short binucleate segment of mycelium; 1*a*, branching tip of hypha in very young fruit-body; 2, 2*a*, 3, young hypobasidia showing two nuclei; 4, hypobasidium showing the nucleus in which the eight chromosomes are distinctly present; 5, hypobasidium showing fusion nucleus before the nucleoli fuse; 6, same, showing enlarging fusion nucleus; 7, same, showing the prochromosomes definitely present in the fusion nucleus; basal septum distinct; 8, 9, 10, nearly mature hypobasidia; 10*a*, mature hypobasidium together with a young hypobasidium into which the enlarging nuclei are just migrating; 11, transverse section of the mature hypobasidium; 12, the same showing the peripheral prochromosomes in section; 13, hypobasidium showing the eight distinct chromosomes; 14, transverse section of slightly later condition than fig. 13; 15, hypobasidium showing the two daughter nuclei separated by the first longitudinal septum; 16, hypobasidium in which the two nuclei are dividing simultaneously while four epibasidia are forming apically; 17, hypobasidium showing two nearly mature epibasidia; 18, hypobasidium showing the two nuclei dividing simultaneously before any epibasidia appear; 19, 19*a*, hypobasidia showing two mature epibasidia; 20, group of hypobasidia showing (left coarse epibasidia; 20*a*, hypobasidium collapsing as the elongated nucleus passes into the well advanced spore; 21, 22, 24, hypobasidia showing the development of the four apical epibasidia; 23, vacuolate mature spores at tip of collapsed epibasidia; 25, hypobasidium showing one of the nuclei migrating; 26, nearly mature spore showing large central vacuole and vacuolate condition of apiculus and supporting sterigma; 27, abnormally large spore formed at tip of collapsed sterigma; 28, mature spores showing vacuolate condition and distinct apiculus; 29–33, stages in the development of the germ tube; 34, spore similar to that of fig. 27, showing rather thick, apical germ tube into which the nucleus has just migrated; 35, mature spore showing germ tube at the tip of which the slender sterigma is formed to bear the spore; 36, mature secondary spore still attached to collapsed primary spore; 37, spore germinating by a branching germ tube; 38, structures found in young fruit-body, in hypobasidium containing large fusion nucleus (left) young hypobasidium before nuclear fusion (center). Paraphysis and septate branched hypha (right); 39, binucleate conidia; 40, truncate hyphal tip sometimes found among the basidia.

NORTH AMERICAN HYPHOMYCETES II. NEW SPECIES AND A NEW GENUS

DAVID H. LINDER

(WITH PLATE 50)

Among the specimens of Fungi Imperfecti that the writer has recently had occasion to study, three stand out as quite distinct from any that have been described.

The first of these, communicated by Professor H. S. Jackson of the University of Toronto, grew on the white resupinate fructification of either *Corticium* or *Peniophora*, where it formed a dark brown and somewhat cottony layer. Microscopically the form is unique among the phragmosporous forms of the Dematiaceae. The type of branching of the conidiophores is strongly reminiscent of that seen in the genus *Botrytis*, while the method of spore formation is so different from anything found in near-by genera that it is somewhat difficult to place the form in the proper tribe. It approaches *Spondylocladium* most closely since the spores occur in whorls around the upper part of the terminal and subterminal cells of the main axis and branches, but it differs from that genus since, instead of producing the conidia in whorls and directly upon the simple main axis, the conidia are produced singly on short, simple, one-celled branches that are formed verticillately (PLATE 50, FIG. 1, 3). It is this arrangement that suggests that the species should be placed in the form-tribe Acrothecieae, yet in this group it is somewhat anomalous since the genera of this tribe are characterized, in addition to the verticillate arrangement of the conidia, by the simple erect conidiophores, whereas the species under discussion is ramose, and the conidia are not attached directly to the main axis of the conidiophore. Therefore, because of the striking characters that this fungus presents, a new genus and species is proposed.

[1] Contribution from the Laboratories of Cryptogamic Botany of Harvard University, No. 129.

Spondylocladiella gen. nov. PLATE 50, FIG. 1–3

Fungus conidiophoris rectis vel adscendentibus, ramosis et sursum ramulos simplices verticillate vel subverticillate gerentibus; conidiis in ramulis acrogenis, unicis, fuscis, ellipsoideis, 2-(raro 1- vel 3-) septatis.

Condiophores erect or ascending, branched and bearing verticillately or subverticillately on the upper part of terminal or subterminal cells, short, one-celled branchlets; the conidia solitary and acrogenous on the branchlets, fuscous, ellipsoid, 2- (rarely 1- or 3-) septate.

Spondylocladiella botrytioides sp. nov.

Coloniae effusae, breve bombycinae, atro-fuscae; conidiophoris 300–400 × 5–9 μ, rectis vel adscendentibus, septatis, infra hyalinis vel subhyalinis, sursum fuscis pellucidisque, ramosis, ramis plerumque unilateralibus nonnumquam alternis, cellulis terminalibus vel subterminalibus ramulos unicellulares verticillate vel subverticillate gerentibus; ramulis subhyalinis vel dilute fuscis, 8–12 μ longis, 4–7 μ diametro; conidiis in ramulis solitariis, acrogenis, sessilibus, ellipsoideis vel inaequilateraliter ellipsoideis, fuscis, 12–20 × 7.5–9 μ, (1)—2-(3-) septatis, leniter ad septa constrictis.

Colonies effuse, short-cottony, dark brown. The conidiophores 300–400 × 5–9 μ, erect or ascending, branching above, septate, the lower cells hyaline or subhyaline, the upper cells light fuscous and pellucid, the branches mostly unilateral or occasionally alternate; the terminal or subterminal cells bearing 1-celled branchlets verticillately or subverticillately; the branchlets subhyaline or dilute fuscous, 8–12 μ long, 4–7 μ in diameter. The conidia solitary, acrogenous, sessile, on the 1-celled branchlets, 12–20 × 7.5–9 μ, (1-) —2- (3-) septate, slightly constricted at the septa, ellipsoid or inequilaterally ellipsoid, fuscous and more deeply colored than the conidiophore.

Growing on the hymenial surface of *Corticium* (?). Toronto, Canada, December 26, 1931. H. S. Jackson, *3199*, type in Farlow Herbarium and the Herbarium of the University of Toronto.

The second species to be described is a very pretty one which was found in the greenhouse growing on flower pots made from tobacco stems. This species, growing in association with *Oedocephalum* sp. and occasionally on the ascocarps of *Ascobolus* sp., formed no conspicuous colonies and was evident only as scattered conidiophores or small tufts. A microscopic examination showed that it belonged in the genus *Dactylaria,* near *D. Orchidis* and *D. candida,* but it differed from these in the size and the number of septations of the conidia. To indicate its relationships, a key

has been compiled from the descriptions of the known species as given in the various volumes of Saccardo's Sylloge Fungorum. With the exception of *D. simplex* (Pr.) Sacc. and *D. graminum* (Schw.) Sacc. for which no measurements are available, all species are to be found in the key which follows:

1. Conidia over 30 μ long .... 2.
1. Conidia less than 30 μ long .... 6.
2. Conidia less than 12 μ diameter .... 3.
2. Conidia more than 12 μ in diameter .... 5.
3. Conidia 4-guttulate, then 3-septate, the conidiophores aurantiaceous *D. Orchidis* Cke. & Mass.
3. Conidia 4–8-septate .... 4.
4. Conidia 4–5 septate, 45–56 × 7–9.5 μ — *D. candida* (Nees) Sacc.
4. Conidia 5–6–(8)-septate, 27–45 × 8–11 μ — *D. pulchra* Linder.
5. Conidia 5–6-septate, 45 × 15 μ — *D. oogena* (Mont.) Sacc.
5. Conidia 6–7-septate — *D. oogena* var. *Pancierii* Sacc.
6. Conidia 18–22 × 7–9 μ — *D. parasitans* Cav.
6. Conidia less than 7 μ in diameter .... 7.
7. Conidia 8–10 × 2.5–3 μ — *D. mucronulata* Ellis & Langl.
7. Conidia more than 12 μ in length .... 8.
8. Condia 20–25 × 4 μ; apex of conidiophore often spatulate inflated *D. purpurella* Sacc.
8. Conidia 16–26 × 4–5 μ; apex of conidiophore not inflated *D. echinocephala* Massal.

It can be seen from the above key to species that this one is quite distinct and hence the name *Dactylaria pulchra* is proposed.

Dactylaria pulchra sp. nov. PLATE 50, FIGS. 4–5

Hyphis sterilibus in substrato immersis; conidiophoris erectis, 1–3-septatis, 100–175 × 3.5–4 μ leniter fastigatis, hyalinis, simplicibus vel ad apices breve ramosis; et sterigmates terminales et laterales gerentibus conidiis (27)–34–40(45) × (7)–9–11 μ, hyaline vel dilute roseis, oblongo-fusoideis, (4–)–5–7–(8–)-septatis.

Sterile hyphae growing in the substratum. The conidiophores erect, one to three-septate, 100–175 × 3.5–4 μ, slightly tapering upwards to 2–2.5 μ, hyaline, simple or short-branched at the apices and bearing terminal and lateral sterigmata on the somewhat zigzag terminal or subterminal cells. The conidia (27)–34–40–(45) × (7)–9–11 μ, hyaline or dilute rose colored in mass, oblong fusoid and occasionally slightly curved, (4–)–5–7–(8–)-septate.

Growing in a greenhouse on flower pots made of tobacco stems.

Cambridge, Massachusetts, December 1933, *D. H. Linder,* type, deposited in the Farlow Herbarium, Harvard University.

The third species to be described belongs in the dark-spored group of the dictyosporous Tuberculariaceae. It is characterized by the pulvinate or irregularly hemispherical sporodichia upon which chains of spores are formed in basipetalous succession. The spores, at first dilute fuscous and sparsely warted, are transversely septate, with maturity become conspicuously and rather closely warted, nearly black and opaque, and both transversely and longitudinally septate. These characters place the species in the genus *Bonordeniella,* but since the conidia are irregularly ellipsoid rather than irregularly globose, as is true of *B. memoranda* Penzig & Sacc.,[2] the type and only species hitherto known, it becomes necessary to apply to the American species a new name, and because of the rough warty appearance of the spore, *Bonordeniella aspera* is proposed.

Bonordeniella aspera sp. nov. PLATE 50, FIGS. 6–8

Sporodochia atro-brunnea fere atra, solitaria vel gregaria, interdum coalescentia, irregulariter hemisphaerica vel lobulata, usque 1.5 cm. diam., extus ruguloso-pulveracea; conidiophoris 20–40 × 2–3 μ, infra hyalinis vel subhyalinis superne fuscescentibus; conidiis ellipsoidesi, 19–28 × 7–11 μ, atrofuscis, primum transversa septatis deinde transversa et longitudinaliter septatis, leniter ad septa constrictis, verruculoso-asperatis, in catenulas simplices digestis, catenulae saepe cellulis minoribus hyalinisque interruptae.

Sporodochia dark brown, almost black, solitary or gregarious, occasionally coalescing, irregularly hemispherical or lobulate, up to 1.5 cm. diam. or larger by fusion, externally rugulose-powdery; conidiophores 20–40 × 2–3 μ, hyaline or subhyaline below becoming darker above; conidia ellipsoid, 19–28 × 7–11 μ, deep fuscous, opaque, at first transversely septate becoming transversely and longitudinally septate, slightly constricted at the septa, warty roughened, formed in simple chains, the chains often interrupted by small hyaline cells.

Haverhill, Massachusetts, on well weathered fence-post (probably oak), Oct. 29, 1933, *S. K. Harris.* Type deposited in the Farlow Herbarium, Harvard University.

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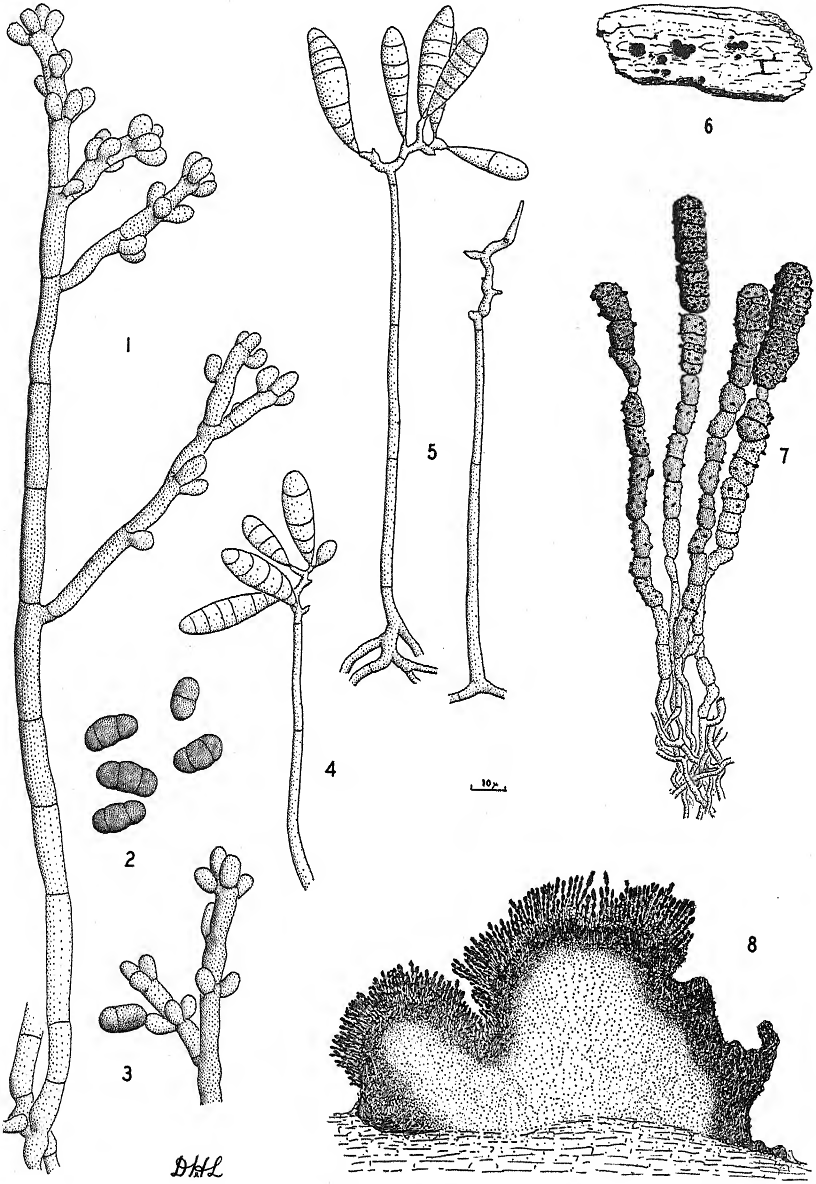
[2] Penzig, O. & P. A. Saccardo, Fungi Javanici p. 116, *pl. 80, fig. 4,* Leiden 1904.

EXPLANATION OF PLATE 50

All drawings except figure 6 were made with the aid of a camera lucida from material mounted in lactophenol and with the exception of figures 6 and 8 are reproduced at a magnification of $\times$ 500.

Fig. 1–3, *Spondylocladiella botrytioides* showing the characteristic type of branching of the conidiophores, the verticillate or subverticillate arrangement of the 1-celled sporogenous branches, the attachment of the conidia, and the variations in the shape, size, and septation of the conidia; 4–5, *Dactylaria pulchra* showing the typical conidiophores and manner in which conidia are produced; 6–8, *Bonordeniella aspera,* fig. 6 depicting the habit of the fungus, natural size; 7, shows the conidiophores arising from the stromatic tissues and bearing chains of spores, some of which have reached maturity and are deep brown, opaque and transversely and longitudinally septate. The chains are occasionally interrupted by small hyaline cells; 8, shows a section through the stroma, the context of which is hyaline within, becoming fuscous towards the surface from which arise the conidiophores. $\times$ 50.



HYPHOMYCETES

MYCOLOGICAL LETTERS FROM M. A. CURTIS 1856–1861

Neil E. Stevens

The herbaria accumulated in this country prior to the war between the States were largely the work of men who were amateurs in the best sense. Even the few who held professorships or curatorships were in constant communication with workers who collected, preserved, and exchanged plant specimens as a pastime and with the zeal now characteristic of stamp collectors. An intimate picture of the manner in which the enthusiasm and energy of the tyros was directed by the more experienced workers is found in the letters of M. A. Curtis to the Rev. Joseph Blake. These letters form a part of Blake's botanical correspondence and are preserved with his herbarium in the University of Maine. For the privilege of examining this interesting collection and of publishing the letters here quoted the writer is indebted to the University of Maine and particularly to Professor F. H. Steinmetz of that institution. I am also indebted to Mr. Arthur H. Norton, Secretary of the Portland Society of Natural History, for the following brief biographical sketch:

"Joseph Blake was born in Otisfield, Maine, January 20 1814, and died at Andover, Massachusetts, May 6, 1888. He was graduated from Bowdoin College in 1835 and Bangor Theological Seminary in 1840. Ordained as Pastor at Cumberland, Maine, in 1841; he served there until 1859. He was Pastor at Gilmanton, New Hampshire, from 1860 to 1878, and resided at Andover, Massachusetts, from 1878 until his death in 1888. He married a lady from Wells, Maine, hence the large collections of plants which he made in that town. He was a very active collector, making a goodly herbarium, largely from the towns of Cumberland and Wells, Maine; and Gilmanton, New Hampshire. His own collection, after his death, was purchased by friends and presented to the Maine State College, now the University of Maine, at Orono. During his life he contributed

generously to various herbaria, including this in Portland. Blake published very little though he was responsible for the list of Graminiales in Goodale's Catalogue of the Plants of Maine published in the Proceedings of the Portland Society of Natural History I, (1) and (2), 1862 and 1869. Mr. Blake's coauthorship appears in 1869 in the second part."

References to Blake's collections are found in the papers of several botanists and accounts[1, 2] of his two ascents of Mount Katahdin, taken from manuscripts prepared for the entertainment of his children, were published by Mr. Norton in the Maine Naturalist. On the second trip he discovered *Saxifraga stellaris*.

Curtis' mycological work is too well known to need review and biographical sketches[3] are available.

THE OPENING OF THE CORRESPONDENCE

The correspondence between Curtis and Blake, like many similar ones, began by a sort of "Letter of Introduction" from Asa Gray. Blake after collecting flowering plants for some years had become interested in fungi and received the following reply to an inquiry for sources of further information.

CAMBRIDGE, Oct. 13, 1855.

Dear Sir:

Just home from a hurried & sudden visit to London and Paris, I find your favor of Sept. 8th.

Publications on Fungi—such as you want—there are none for N. Amer. On this subject you should correspond with Charles J. Sprague, Esq., Boston, who has lately taken up the subject, and especially with Rev. Dr. M. A. Curtis, Society Hill, South Carolina. He will name the fungi you send him.

I have *Saxifraga azoides* from Willoughby Mt., but not *S. Aizoon*. Send me a leaf from yr. specimen, and I will tell you if it is the plant—which will be new for New England, & will go in new edition of the Manual.

Excuse great haste

Yours truly,

A. GRAY.

[1] Blake, Joseph. An Excursion to Mount Katahdin. Maine Naturalist **6**: 71–73. 1926.

[2] Blake, Joseph. A Second Excursion to Mount Katahdin. Maine Naturalist **6**: 74–83. 1926.

[3] Shear, C. L. and Stevens, N. E. The Mycological Work of Moses Ashley Curtis. Mycologia **11**: 181–201. 1919. And other papers therein cited.

Those were leisurely days and no doubt the life of a country clergyman in Maine in the middle of the last century held other than botanical activities. It was apparently over a year later that he wrote Curtis and in reply received the following very encouraging letter:

SOCIETY HILL, S. CAR.; Oct. 30th, 1856.

Dear Sir:

Yours of the 17th rec'd this morning, could hardly have reached me at a more unlucky moment than the present. I am very much pressed for time & do not expect much leisure for botanical studies during the remainder of the present year. Nevertheless, to show my good will, & to encourage you in further investigations in the interesting but obscure field of Mycology I have made a hurried examination of your specimens, & report as well as I can under the circumstances on next leaf. I have also selected a few specimens, for you, taken (all but one) from letters just rec'd from other regions & now lying before me. These will, so far, help to furnish material for knowledge in this much neglected department of science.

I have no correspondent in Maine, though I occasionally receive specimens from that State, & shall be very glad if you continue to send me specimens of Fungi. I will do what I can towards facilitating your study of the Fungi. You must, however, bear in mind, that, among objects so obscure & so little known, I can not be expected to know everything at sight, & so may not be able to gratify your curiosity in all cases until after long delay. I have had species for many years in the hands of the best European Mycologists, & have not yet rec'd their decisions. Besides, my correspondents are numerous, & it is no easy matter to attend to all. Within a week I have rec'd over 600 specimens of Fungi. Remember too that most of the species have to be subjected to the Microscope. Under such circumstances you will find cause to have patience with me. But I will do my best, & hope I shall be able to satisfy you on the whole.

What books, & what sort of microscope, have you?

I take the liberty of sending a poison mixture, which you will find a sine qua non, if you would preserve your specimens from insects.

	Corrosive sublimate	Z iv
	Sulphuric ether	Z iii
Dissolve & add		
	Alcohol	Z iii
	Spt.s of turpentine	Z ii

The thinner species should be washed with this mixture by means of a feather. The larger fleshy & woody forms, after being dried, should be more thoroughly washed or even saturated within it & then dried again. Otherwise, they will inevitably be destroyed by larvae and insects.

In much haste

Yours respectfully,

M. A. CURTIS

Still another year elapsed and another letter was received from Curtis before the sending of specimens began.

HILLSBOROUGH, N. CAR. Sept. 1st '57

Dear Sir,

Last October I recd a letter from you enclosing specimens of Fungi, and I immediately replied, giving names of the species. I judged from your letter, that you were anxious to prosecute the study of these obscure and much neglected productions, and were desirous of such aid as I can give. But having heard nothing further from you, I have thought that a letter from one or other of us has miscarried, and hence that you may have thought me unwilling to continue the service required. I take the liberty, therefore, of assuring you that I shall be very glad to receive specimens from your State, as it will serve to increase my knowledge of the range of species, and will very likely bring to light some things before unknown. In assisting you, therefore, I shall be benefiting myself, and I shall very cheerfully determine species for you as far as I am able. From Connecticut, Massachusetts, and N. Hampshire, quite a number of new species, and of species before unknown to me as American, were sent to me last year. From Maine I have none but a few from the vicinity of Portland, besides those you have sent. I shall be well pleased if you can take such interest in these things as will induce you to persevere in their collection.

Very respectfully

M. A. CURTIS.

Following the actual sending of specimens there came from Curtis more specific directions for collecting and preserving specimens, a list of the best books on fungi and suggestions for securing and equipping a microscope. The letters covering these points are here published without correction or alteration except the omission of occasional " lists " of identifications. They furnish a picture of the methods of work of this pioneer American Mycologist more detailed in some respects than any hitherto available.

HILLSBOROUGH, N. CAR. Sept. 20, 1857.

Dear Sir.

This morning I recd. yours of the 16th and on last page you have report upon the enclosed species. Two of them are perhaps new. Though you should not make a vigorous study of these things, you may do good service to science by collecting whatever falls in your way. American Mycology is already indebted to you, as you see above, for some additions to her stores. Last year Mr. Sprague of Boston, & Ch. Wright of Conn. added about 50 new species to my stock, from N. England.

As to Books, you need Systema Mycologicum, (3 vols.) Elenchus Fungorum, (1 vol.) & Epicrisis Syst: Mycol: (1 vol.) by Fries. These will cost about $14.00, & can be had through any importing Bookseller of Boston, I presume Bailliére of N. York is most familiar with works of this

kind, & I have always found him prompt, & reasonable in his charges. Having houses in London, Paris, Madrid, &c he has unusual facilities for accommodation.

You will find Payer's Botanique Cryptogamique very serviceable, which is illustrated with beautiful figures. Cost about $3.00.

Berkeley's "Introduction to Cryptogamic Botany" will be useful as an *Introduction,* & has some figures of Fungi, a goodly number of them American species. Price $5.00.

In advising you in regard to a Microscope, I must first know how much you are willing to expend for an Instrument. A suitable Compound Microscope can not be had under $75. If you can spare $100. I should advise one of Robbins' Instruments at that price. But if your means are too small for such an outlay & for such a purpose, as is generally the case with country Clergy, I should recommend one of Chevalier's Doublets. I have never used any thing else, & I can see with it objects measuring only 1/10,000 in. in length. I happen to have a spare one, Mr. Berkeley having presented me with one. If you will run the risk of its transportation by Mail, & give me what it cost ($7.00) I will let you have it, as I have no use for two. I use it in the stand of a small Compound Microscope which cost $10. If you have nothing of the sort, you can have a stand made for it, of which I will give you the idea. The Doublet is about an inch in diameter, & 2 lines thick, & can go by mail easily enough.

Fleshy Fungi are preserved by pressure in paper like Flowering Plants. But I let them become flaccid in the air before subjecting them to a gradual pressure. The paper must be changed frequently at first. It is rather troublesome & specimens are often badly injured by Larvae before they can be dried. They are so much changed by the process, that the essential characters can not always be gathered from dried specimens. It is therefore necessary to write out full descriptions from the fresh specimens. And as the dried specimens are eagerly sought by insects, they must be poisoned as soon as dried, or they will soon be consumed.

[Here follows a list of fungi]

In sending again, do not repeat your Numbers, but continue on from 14. Otherwise future references, (which will be necessary in case of species not at once determinable) will not be intelligible.

Always give the name of the object on which the Fungus is,—the specific name when possible.

The important notes to be made of the fleshy Fungi are the *dimensions* of Cap & Stipe:—Color of the different parts:—whether the Gills are *free* from the Stipe or attached to it:—if the Stipe be solid, hollow or fistulous,—*color* & *taste* of the juice, if any;—& *odor* of the plant, when describable.—A very essential character is the *color* of the *Spores.* These are procured by laying the specimens (gills or pores downward) for a few hours upon white or blue paper. These Spores should be sent with the specimens to be glued with them upon the tickets. The fuller your descriptions the better. It is quite impossible to determine species often times without them;—& with them, such is the number of these things, it is often difficult. It is only when we are pretty familiar with a species, that we can determine it without notes. If you could also give full drawings of the specimens, it would be a great help. Sprague sends me very fine sketches.

My poison mixture, which I have found very effectual, is composed of

Corrosive Sublimate—Z IV
Sulphuric Ether —Z III
Dissolve & add
Alcohol —Z III
Spts. Turpentine —Z II

On thin specimens I generally apply it with a feather. Upon large & especially the woody species I usually pour it so as to saturate at least all the surface. These liquids are so volatile, that specimens dry very quickly after the application, & may be put almost immediately in the Herbarium. Bulky forms are of course put on shelves in a Cabinet.

Unless you have a pretty large collection, say 100–300 of bulky specimens, it is hardly worth while to send me a package at present. By next Fall, you may perhaps have enough, when I will give directions for forwarding; or in next letter, if you wish to send soon. In the mean time, you can send your smaller forms by letter.

In the matter of the Doublet which I offer you, the proposition is made supposing that you will not easily find one in this country. If you could get one in Boston, of course it would be better to do so.

Respectfully yours,

M. A. CURTIS.

HILLSBOROUGH, N. C. Nov. 9th, 1857.

Dear Sir,

Herewith please find report on your last two envois. The specimens have been examined under the pressure of other duties, & a portion of them are not in very good condition;—but most of them are given with confidence. I was anxious to report them before entering upon a journey which I am about undertaking to Alabama, as I desire, upon my return in a few weeks, to come with free hands upon a large collection of Cuban Fungi just sent me by C. Wright. These will keep me busy for a while, so that I shall hardly be able to do much more for you before Jany. next. This, however, need not prevent your sending in the meanwhile, if you choose. I will examine your specimens as soon as I can.

Such Genera as *Sphaeropsis, Septoria,* &c. have so many forms, & are perhaps only secondary states of other Genera, that it is an empirical business to impose names upon them. *Pestalozzia* & *Phoma* form many uncertain species. Hence you see no specific names of these in the list.

In haste—yours respectfully

M. A. CURTIS.

[No date]

Dear Sir,

I have managed to find time to put up a few Fungi by way of support to the Doublet, & to make a rough kind of sketch of my Doublet stand,—all which are herewith enclosed. You had better ascertain what the maker will charge for constructing such a stand, before making a bargain with him. Those not accustomed to this sort of manufacture might charge \$10.–\$15. A Boston man, & the only one I could hear of in the City, six years ago,

who could work in brass, would not undertake such a thing for me under the latter sum. You can buy such a stand (the upper arm only altered to receive the Doublet) with all the appurtances of a Compound Microscope for \$8.–\$10.—Mine is of that kind. The thing I have sketched should not cost over \$4 or \$5.

The Doublet has a focal distance of not more than one line. The glass slides I use I have generally made myself out of bits of clear window-glass, cutting them about 1½ in. long & ½ in. wide.

A small bit of the hymenium of the Fungus is taken with the point of a sharp knife & macerated in a drop of water upon the slide—mashing the piece so as to be sufficiently disintegrated & mingled with the water that the mass may pass under the lens without any portion of it touching the lens. The field of vision with this Instrument is so limited, that it is only suited to the examination of objects invisible to the naked eye. It requires a firm stand & a nice adjustment.—I have given you a view of the fruit of Peziza ollaris, as it appears under this Doublet, magnified about 250 diameters. You could not see this fruit at all with the naked eye. I have never used any instrument but this.—You must always keep it protected from dust when not in use. This would gradually scratch & deface the Lens. If anything gets upon the lens, wipe it with a soft silk handkerchief free from dust, having previously blown off whatever the breath can remove.

Very truly yours,

M. A. CURTIS.

THE LAST LETTER

A number of the Curtis letters in the Blake correspondence are omitted because they add little to the knowledge already in hand regarding Curtis. It appears fitting, however, to include the closing paragraphs of the letter with which the correspondence itself ended and which shows that however little Curtis talked or wrote regarding politics [4] he was in that as in other respects a close observer.

HILLSBOROUGH, N. C. Feb. 21 '61.

Dear Sir:

. . . .

It is interesting to read your notes of a Maine Winter. I was raised in the Western part of Massachusetts and know very well what snow and ice and a low temperature are; but having now enjoyed a sub-tropical climate for 30 yrs. I have no desire to try a boreal Winter again. We have as cold weather here as I can enjoy, for we can freeze to death in Carolina, though not quite so quickly, as in Maine. Four years ago the thermometer here stood at 16° below Zero and snow (several feet deep) lay for weeks on the ground. But that was extraordinary. This winter we have had but one snow, and no ice has formed for packing away. This again is unusual,

[4] See also in this connection—Stevens, N. E., Two Southern Botanists and the Civil War. Scientific Monthly **9**: 157–166. 1919.

for we generally put up ice here for summer use. Violets, crocuses, & hyacinths are now blooming in my yard, & the elms are beginning to put out their blossoms.

You allude very delicately to the political state of affairs but I do not care to respond or correspond on that distressing subject. The all-wise ruler chastises as well as blesses, & I am sadly afraid that we are to be terribly afflicted for our national sins of worldliness, boastfulness, self-reliance & various acts of injustice. The 2nd Psalm and the Sermon on the Mount are what I generally recommend now for reading, more than ever before, & more thoughtfully. A very few days will, I suppose, bring matters to a crisis. If the incoming administration attempts coercion, whether it be legal & right or not it will unite the whole South as one man in resistance. The only possible chance of a restoration of the Union is just to let the seceding States alone. A little spark now thrown into such combustible material as the South presents, will kindle a blaze the like of of which was never before seen. Is not almost any sacrifice desirable that will avert the catastrophe. The Border States, so called, of the South, i.e. all the Slave States north of the seceded ones, have a large majority in favor of preserving the Union, & yet one impolitic act of Pres't Lincoln will throw them all out. God help us."

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WASHINGTON, D. C.

FUNGICIDAL VALUE OF SOME COMMON DYES AGAINST DERMATOPHYTIC FUNGI

ADELIA McCREA

During the past twenty years, a considerable amount of work has been done relative to the value of dyes in bacteriological and, more recently, in mycological studies on the prevention or control of growth of the organisms concerned. In order to correlate the results, it seems desirable to give a brief resumé of the purposes and findings of some of these various workers.

In 1912 Churchman (3) published an extensive study on the selective bactericidal action of gentian violet, comprising over 300 strains of 137 species. He found the gentian violet positive and negative reactions far more definite and constant than reactions to the Gram stain but concluded that the effect on growth should be considered bacteriostatic (a term which he seems to have introduced) rather than bactericidal. Two years later, 1914, Krumwiede and Pratt (7, 8) studied 30 strains, covering all types of pathogenic bacteria, in relation to 40 samples of dyes, stressing especially the green dyes. They confirmed Churchman's findings as to the inhibition of most Gram positive bacteria but the Gram negative organisms grew freely as a rule. However, several of the green dyes showed marked selective action among the typhoid-colon group, suggesting their usefulness in enrichment of the typhoid-paratyphoid species. Kligler (6) in 1918 reported the inhibiting effect of 28 compounds on 2 Gram positive and 7 Gram negative organisms. As was true for the previous workers, he found the most marked selective action in the dyes of the triphenylmethane group, such as gentian violet, malachite green, etc., which are much more toxic for the Gram positive organisms than for Gram negative types. Ten years later, 1928, Mallman, Thorp and Semmes (12) tested gentian violet, basic fuchsin, crystal violet, acriflavine and brilliant green for selective action in paratyphoid

types but found only brilliant green to be satisfactory. They stressed the importance of the *source* of the dyes used, as great variation is found in products from different manufacturers. On this point the writer is able to confirm their statement and extend it to the work on fungi reported herein.

Several contributions have been made on the *fungicidal* potency of aniline dyes. After gentian violet had been shown to be so active against Gram positive bacteria, Farley (5) in 1920 tested its usefulness as a restraining agent in his work on isolation of mold pathogens. He reasoned that if the many Gram positive bacteria could be inhibited by a dye that would permit normal mold growth, isolation of pure species would be facilitated. He found that, although molds in general are Gram positive, they are much more resistant to gentian violet than are Gram positive bacteria. Thus he was able to grow normal cultures of 50 species of *Actinomyces, Sporotrichum* and "ringworm" fungi on media containing sufficient gentian violet to inhibit troublesome bacterial growth. Castellani (1, 2) 1928, 1929, reported the use of a solution of carbol-fuchsin in combatting certain types of skin infection by fungi. In most of his cases the responsible organism was not isolated, or at least is not given, but he has had considerable clinical success with this dye. The writer has been unable to confirm *in vitro* or on guinea pig lesions any appreciable value for this formula against *Epidermophyton rubrum.* Coons (4) 1927 and Leonian (9) 1929, found that malachite green decidedly inhibits development of *Fusarium* species, the latter having tested this dye upon 220 different cultures of *Fusarium,* only three of which made any growth at 1–10,000 while many were inhibited at 1–500,000. Leonian (10) also in 1930 used malachite green as an aid in studying *Phytophthora* species. He records a grouping of the strains by means of their differential growth upon media containing malachite green in dilutions up to 1–16,000,000 and holds that, despite the difficulty due to borderline reactions the dye is nevertheless useful in defining groups of species or strains within the genus *Phytophthora.* In 1932 Leonian (11) again tested the fungistatic potency of malachite green, and crystal violet as well, on 26 cultures of skin fungi of the genus *Trichophyton,* his object being mainly to study the effect of position of inoculum. Despite

uniform conditions, results were quite erratic and the conclusion was drawn that such dies are of doubtful value in identification work with *Trichophyton* types.

For several years the writer has been working with species of the four chief dermatophytic genera—*Trichophyton, Epidermophyton, Microsporum* and *Achorion*—in an attempt to find an ideal fungicide. Many substances have been tested for both fungistatic and fungicidal properties but the work herein given concerns only three species of fungi and a group of five of the most significant aniline types tested.

So far as the *fungistatic* power of these dyes is concerned, it may be disposed of briefly because in no case was the fungistatic limit widely separated from the fungicidal. In marked contrast to results with bacteria, it appears that unless a substance is capable of killing the fungus (fungicidal) it does not exercise any marked antiseptic effect. This was determined by sub-culturing, at suitable intervals after planting by fungistatic method, to determine whether the inhibition was merely stasis or whether killing action had occurred.

The fungicidal test method used in this work was published by the writer (13) in 1931, hence will not be given here in detail. Suffice it to say that painstaking effort is exercised to secure uniformity of physical conditions, medium, reagents, spore suspensions and all other factors capable of standardization.

The pathogenic organisms used were *Trichophyton interdigitale* Priestly and *Epidermophyton rubrum* Castellani. As a check, *Aspergillus niger* van Tiegham was used because it is usually quite resistant to inhibiting substances—which may account in part for its status as a "laboratory weed." The table shows the relative susceptibility of these organisms to the dyes tested. Several points are apparent from the results which may be summarized as follows:

1. The "green dyes," malachite green and brilliant green are greatly superior in potency compared with any others studied.

2. These two dyes are of equal value as fungicides.

3. They show decidedly a selective action on the organisms tested, *A. niger* and *E. rubrum* being considerably more resistant than is *T. interdigitale*.

4. Selective action is also shown by aniline violet which in concentrated solution kills *T. interdigitale* in 5 minutes but fails to kill *E. rubrum* or *A. niger* in intervals up to and including an hour.

5. Neither gentian violet nor basic fuchsin show any significant fungicidal power against these three organisms, in any practicable dilution.

COMPARATIVE FUNGICIDAL VALUE OF FIVE COMMON ANILINE DYES

	Aniline violet	Fuchsin basic	Gentian violet	Brilliant green	Malachite green
Trichophyton interdigitale............	1–500 Kills in 5 minutes.	Does not kill.	Does not kill.	1–75,000 Kills in 1 minute.	1–75,000 Kills in 1 minute.
Epidermophyton rubrum............	Does not kill.	Does not kill.	Does not kill.	1–10,000 Kills in 1 minute.	1–10,000 Kills in 1 minute.
Aspergillus niger....	Does not kill.	Does not kill.	Does not kill.	1–10,000 Kills in 1 minute.	1–10,000 Kills in 1 minute.

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LONGEVITY OF MERULIUS LACRYMANS IN WOOD DESTROYED BY ITS GROWTH

Adelia McCrea

This note pertains to the persistence of life of *Merulius lacrymans* in wood destroyed by the growth of the fungus. Perhaps it should be stated at once that, although this species is herein termed *M. lacrymans* in the sense of American usage, it is doubtless *M. americanus* according to Burt's (1) classification.

In April, 1928, two specimens were received for study: (*a*) a piece of wood from the body of an automobile and (*b*) a fungus growing on the cloth upholstery of the same car. The car was one of the finest makes and had been in use between four and five years. It was involved in only a minor collision, but the body crumbled, due to destruction of the wood under the cloth.

At that time, the "mold" stage of the organism could be obtained readily from both the wood and the sporophore and, under proper conditions, would produce fruiting bodies in a few weeks as would also bits of the wood if first moistened. Each specimen has been kept wrapped in parchment paper on a shelf in the laboratory and tested at irregular intervals, at least once a year. In 1930, both were still viable but since then no culture has been obtained from the fungus on the cloth. Recent attempts included preliminary soaking of the fragments but the sporophore appears to be definitely dead.

The wood, however, never fails to give good results of the mycelial (mold) form and appears to be singularly free from any other organisms. If a piece be crumbled over a moist agar plate, every bit of the dust seems able to grow and in due time, transferred to wood, to form new sporophores.

If we may assume that the fungus was present in the wood when the car was built, the history would cover a period of ten to eleven years for the wood and over five years at least for the sporophore which, however, has been "dead" the last two or

three years of that time. It thus appears that life persists considerably longer in the wood than in the fruit body, when both are in the same unfavorable situation. This would agree with Findlay's (2) statement that difficulty in germinating the spores is due to the fact that workers have used spores no longer fresh. Falck (cited by Findlay) is authority for the statement that Merulius spores may be viable after five years laboratory storage but so far the writer is unable to confirm this finding.

These specimens will be preserved and the history of the wood followed up at intervals in an attempt to learn how long the organism will remain viable.

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A DEVELOPMENTAL STUDY OF A NEW SPECIES OF OPHIODOTHELLA [1]

E. Sophia Boyd

(with plate 51 and 2 text figures)

INTRODUCTION

This organism has been observed growing on the leaves of *Vaccinium arboreum* Marsh, in Georgia and other Southern States, and, being a leaf parasite, it has proven especially productive in a study of the development of a peculiar type of perithecium. The asexual stage begins in middle summer and continues throughout the fall, followed by the development of a perfect stage, with perithecia in the mesophyll of the leaf, beginning the latter part of September. At frequent intervals, the infected leaves have been gathered and sectioned, and the development of the organism studied. Both stages are apparently new and are so described in this paper.

This type of organism has been placed in the Phyllachoraceae of the Dothideales on the basis of having no true perithecial walls, but the ontogenetic studies here show a definite wall which places this in the Sphaeriales. On the basis of examinations of type specimens the writer here transfers the genus *Ophiodothella* from the Dothideales to the Sphaeriales.

METHODS

Leaves were gathered first in late September when still living and on the host. Later they were collected on the ground in damp

[1] This investigation was carried on under the direction of Dr. J. H. Miller to whom the writer expresses her appreciation for the generous assistance anl helpful supervision in this work. Also, she is much indebted to Dr. W. W. Diehl, United States National Herbarium, Dr. F. L. Stevens, University of Illinois, and Dr. D. H. Linder, Farlow Herbarium, Harvard University, who have contributed both material and information relative to this study.

places, at seven to ten day periods, until April, when mature asci were found.

Material was fixed in Flemming's fluid, both strong and diluted fifty per cent with water; form-alcohol; and Bouin's fluid. The Flemming's fluid penetrated poorly, fixing only the epidermal and outer mesophyll cells. It increased the brittleness of the leaf, making sections very difficult to cut. However, the leaves were already very brittle, as in most cases they were dead when gathered. The form-alcohol solution penetrated well, but caused slight plasmolysis of the mycelial protoplasm. After Bouin's fluid there was good penetration with less brittleness than after the other two fixatives.

Sections were cut from paraffin, and were stained with Haidenhain's iron-alum haemotoxylin and counter stained in safranin. Very clear definition of nuclear structures was obtained from the use of the iron-alum haemotoxylin, while the safranin made a good contrast stain for the mycelium.

DEVELOPMENT OF THE IMPERFECT STAGE

The first visible sign of infection in the leaf is a small, yellow spot ranging from 1–5 mm. in diameter. A transverse section of this spot showed the vegetative mycelium running parallel to the epidermis. It becomes densely packed in the mesophyll and epidermal cells as well as in the intercellular spaces. The hyphal cells are large, thick-walled, nearly cylindrical, and uninucleate. The mycelium is very much branched, spreading into the epidermal cells, and there, due to oxidation following exposure to air, the walls turn black and give the appearance of a solid black layer as viewed from the outside of the leaf. They do not coalesce to form a stroma; however the layer might be called a pseudoclypeus.

The conidial potential forms between the epidermis and the palisade cells, and is made up of a tangled mass of hyphae, which do not anastomose, but interweave to form a dense plectenchyma of very minute cells (PLATE 51, FIG. 1–2). The conidiophores arise on the upper side of this plectenchyma, between it and the epidermis. They are multicellular stalks which grow perpendicular to the epidermis, forming a parallel layer that resembles an ascal hymenium (PLATE 51, FIG. 1–2). In some cases, however, the

plectenchyma becomes umbonate in the center. The cells of the conidiophore are uninucleate, and the basal cells are much larger than the upper ones (PLATE 51, FIG. 3). The apical cell is constricted into a narrow beak, resembling, to some extent, the one found by Jones (**10**: *pl. 4, fig. 1*) in *Melasmia acerina* Lév. This tip grows into a long, hyaline, filiform spore, which matures after the epidermis has been ruptured. The conidia range from 28–58 × 2 μ; are continuous, and the cells are uninucleate (PLATE 51, FIG. 4).

The fruiting body is an acervulus and not a pycnidium, because the pycnidium, as described by Dodge for *Schizoparme straminea* Shear (**2**: *pl. 4*), starts as a stromatic ball and the conidiophores arise in the center. In the conidial fruiting body of *Ophiodothella Vaccinii* the conidiophores are borne on the surface of the plectenchyma, and not down in it as in a true pycnidium. There is no definite ostiolar cavity as in the pycnidium that Dodge shows, but there is an aperture formed in the epidermis, due to the swelling and upward growth of the conidiophores. The conidia are cut off from the conidiophores successively as in the pycnidium, and are extruded in enormous masses through the ruptured epidermis.

The leaves of *Vaccinium arboreum* are tardily deciduous, hanging on until late December. Conidial infections continue as long as the leaves are alive.

DEVELOPMENT OF THE PERITHECIUM

Archicarp: The perithecial primordium arises in the center of the mesophyll and originates from one or several coiled archicarps, which, at this stage, are seen in a tangle of vegetative threads, as in *Polystigma rubrum* (Pers.) DC. (**5**: *fig. 160*) and in *Poronia punctata* (L.) Fries (**5**: *fig. 184a*). The archicarp (PLATE 51, FIG. 5) is a multinucleate hypha, which becomes septate and elaborately coiled at the base. It consists of a definite basal portion and a long, slender thread, probably a trichogyne, that grows straight up through the leaf and protrudes through a stoma. In many cases, several archicarps are grouped together and, thus, several trichogynes may grow through one stoma. No antheridium was found, but there is a possibility that the conidia, which mature simultaneously with the development of the coil, are ho-

mologous with spermatia, as found in many of the Hypocreales and Sphaeriales. According to Gwynne-Vaughan (**5**: 131), the more complex ascomycetes have antheridia that become detached and single cells function as spermatia; while on the other hand, probably more primitive forms, including all the Plectascales and many forms in the Pezizales, have non-deciduous antheridia. The conidia of *Ophiodothella Vaccinii* show marked resemblance to the spermatia of *Polystigma rubrum,* which are curved filiform cells, containing single elongated nuclei. A trichogyne is present in both, but no relation has been seen between it and the conidia of the one and the spermatia of the other. Higgins (**9**: 428) shows spermatia functioning in *Sphaerella bolleana.* Thus, it is possible that the spermatia in *Polystigma rubrum* are functional, but this has escaped the notice of observers, and judging from related forms in the Sphaeriales, the conidia of *Ophiodothella Vaccinii* probably at one time possessed spermatia similar to the vegetative cells that now function as conidia.

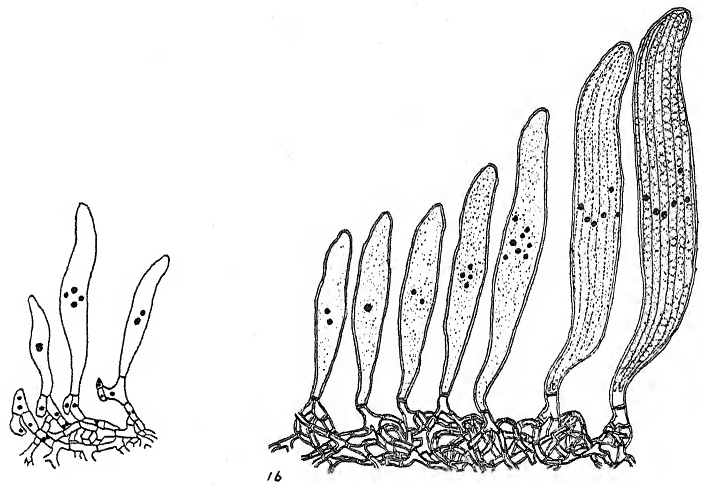
A basal cell in the archicarp continues to enlarge and is seen to possess two very large nuclei (PLATE 51, FIG. 6). This is an oögonial cell in the conception of Gwynne-Vaughan (**5**: *fig. 118–119*). It comes to lie in the center of the developing perithecial primordium. The binucleate condition in the oögonium arises either apogamously by the union of cells in the coil, or from a spermatial nucleus from the apex of a trichogyne.

In the cells of the thread-like trichogyne many very minute nuclei were found. These were so small that they may have been chromatin particles of disintegrating nuclei.

The two nuclei in the oögonium appear to pair and then divide conjugately passing into branches (PLATE 51, FIG. 7) from this cell, in the same manner as Claussen (**6**: 333) found in *Pyronema confluens* Tul., and the writer finds no evidence of a fusion at this point as Harper (**5**: *fig. 108*) observed in *Phyllactinia corylea* (Pers.) Karst., and Gwynne-Vaughan (**5**: 175) in *Pyronema confluens.*

Development of the perithecial wall: Below the oögonial region in the archicarp branch many very fine hyphae arise, completely surrounding the archicarp and proliferating tangentially until a globose perithecial primordium is formed. The peripheral cells are

small and uninucleate. By continued growth a definite wall is laid down (PLATE 51, FIG. 6–7). This is a perithecial wall as defined by Miller (**11**: 194) as being characteristic in the Sphaeriales: ". . . the specialized tissue which arises from the archicarp, and from the beginning encloses the ascigerous centrum." This same idea of a perithecium is later set forth by Graff (**7**: 244) when he says: "Properly the perithecium is a product of the gametophytic stalk cells from which the sexual reproductive organs also develop, and is an enclosing protective envelope whose initiation is evidently closely correlated with the formation of these organs."



1a 1b

FIG. 1. a, Development of ascal hooks; b, Nuclear divisions in an ascus and formation of eight ascospores.

The wall cells, expanding peripherally, produce a rupture in the center. The inner wall cells now become very much flattened tangentially, and give rise to many sterile threads, which (PLATE 51, FIG. 8) grow toward the center of the perithecium. They are thin, thread-like, branched filaments with free ends, and so could definitely be called paraphyses.

The points of the perithecium facing the two epidermal layers now grow outward, the cells proliferating until the epidermis is ruptured on both sides. Thus two definite ostiola are formed, and the paraphyses developing in these beaks are very small and slender

and should be termed periphyses, such as Emmons and Dodge (**3**: *pl. 27, fig. 3*) show for *Microascus trigonosporus* Dodge. This now is a perithecium with a specialized wall, but is peculiar in the possession of two ostiola which permit the escape of ascospores from both sides of the leaf. The two ostiola are not always directly opposite so that several sections of the same perithecium have to be seen to observe both of them.

The only other forms that the writer has seen having two ostiola protruding through both sides of the leaf are *Catacauma biguttulatum* Theissen (**19**: *pl. 6, fig. 1*) and *Diachora onobrychidis* (DC.) J. Müll. (**4**: *fig. 248f*).

In the mature perithecium (PLATE 51, FIG. 8, and TEXT FIG. 2) the equatorial part of the wall becomes much thickened, while the wall of the ostiolar neck is very thin.

Origin of ascogenous hyphae: When the perithecium expands, rupturing the center, the archicarp with many branches coming from the oögonial region, comes to lie along the wall. In all longitudinal sections of perithecia examined, two such systems were found directly opposite each other in the perithecium, giving rise to two groups of asci. The cells coming off of the oögonial region form the ascogenous hyphae. These hyphae (PLATE 51, FIG. 8) are now found in a tangle along the wall and intermingle with numerous paraphyses. Their tips point inward as opposed to those of *Microascus trigonosporus* described by Emmons and Dodge (**3**: 331) as growing radially outward.

The archicarp of *Ophiodothella Vaccinii* gives rise to ascogenous hyphae before disintegrating, instead of disintegrating and then having the ascogenous hyphae arise from the wall cells as described by Blackman for *Polystigma rubrum* (**5**: 214).

Development of asci and formation of ascospores: The ascogenous hyphae branch freely and give rise to hooks (TEXT FIG. 1*a*), the penultimate cell developing into the ascus, in the same manner as shown by Claussen (**6**: 334) for *Pyronema confluens*. The asci form an equatorial belt on the inner wall and point toward the center (TEXT. FIG. 2), as Müller (**4**: *fig. 248f*) shows for *Diachora onobrychidis*. Theissen (**19**: 435) attempts to prove that Müller's conception (**12**: 346) of the origin of an equatorial belt of asci is incorrect, and he maintains that the asci form all over

the lower half of the locule, as in the normal situation, and that it is only later that the floor of the locule grows out into another ostiolum.

The writer has not examined specimens of *Diachora onobrychidis,* but in *Ophiodothella Vaccinii* the asci do arise in equatorial belts, and both ostiola develop simultaneously.

Two nuclei are cut off in the penultimate cell of the ascogenous

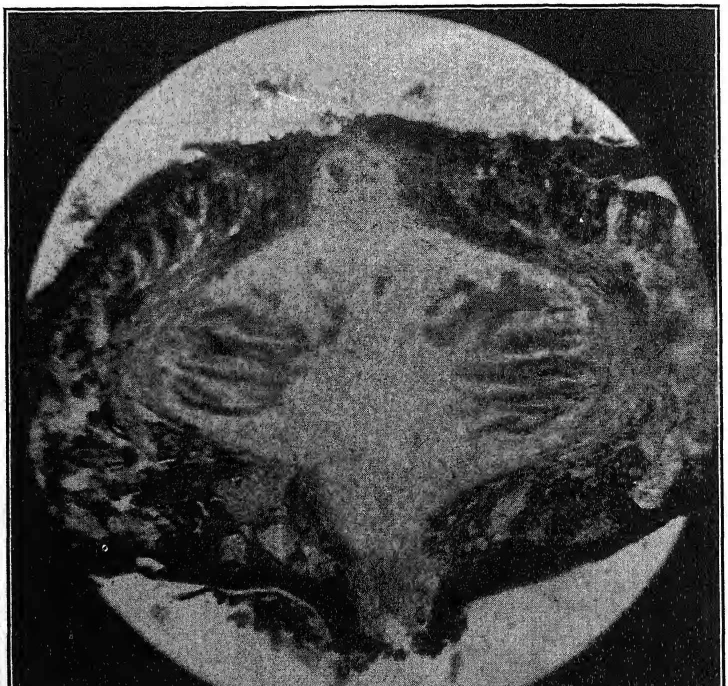


FIG. 2. Longitudinal section through a mature perithecium, showing equatorial belts of asci and two ostiola.

hook, the young ascus growing out toward the center of the perithecium. After fusion, reduction division takes place, followed by a mitotic division, and eight small nuclei come to lie in the center of the ascus (TEXT FIG. 1b). The ascospores are not cut out along lines radiating from a beaked nucleus as shown by Harper (5: *fig. 111*) in *Phyllactinia corylea* and many other Ascomycetes, and

by Schultz (**14**: 310) for *Peziza domiciliana* Cooke. Definite lines running the entire length of the ascus, are laid down. At first these appear to be vascular channels, but as the ascus matures, distinct membranes are formed, delimiting extremely long, filiform spores with one nucleus in the center of each. The ascospores are extruded through a small pore at the apex of the ascus.

TAXONOMIC RELATIONS

This organism has been placed in various herbaria of the country under the name of *Rhytisma Vaccinii* (Sw.) Fries: vide Ravenel Fungi Am. Es. *759,* Fungi Carol. *52,* Ellis, N. Am. Fungi *673* and de Thümen's Myc. Univ. *660.* This was due to a mistaken interpretation of *Rhytisma Vaccinii.* The Schweinitz type, according to Diehl in litt., is a true *Rhytisma* and is not a pyrenomycete. This mistake has probably been due to the fact that *Ophiodothella Vaccinii* forms the conidial stage with a macroscopic appearance very much like *Rhytisma punctatum* (Pers.) Fries. Also the perithecia here do not develop until spring, and on dead leaves lying on the ground, and therefore, probably have been overlooked.

The systematic position of this fungus lies within the Sphaeriales. The reasons for this are that here there are perithecia in the strict sense of the word. There is a specialized perithecial wall arising from the initial coil, definite ostiola with periphyses, and definite paraphyses with free ends. On the other hand the separation characters of the Dothideales are one or more locules in a stroma with no specialized wall enclosing the ascigerous centrum, no ostiolum, only a break in the upper portion of the stroma, no periphyses, the asci growing up in pseudoparenchyma or among threads attached to the roof of the cavity as well as the base.

The correct family within which this fungus should be placed cannot be determined at this time. The family separations in the Sphaeriales, according to Lindau (**4**: 386–387), are based on the position of perithecium relative to substrate or stroma, and amount and kind of stroma, all of which are environmental characters which can be brought forth within any one species. The writer will temporarily place *Ophiodothella* within the Clypeosphaeri-

aceae, because of the presence of a stromatic clypeus within the epidermis, and will await further revision of the order.

The genus *Ophiodothella* Höhn. is based on the species *Ophiodothis atromaculans* Henn., material of which has been loaned the writer by D. H. Linder. This genus is now in the Dothideales according to Theissen and Sydow (**19**: 611) and according to Shear and Clements (**16**: 293). Sections of this material show perithecia in the center of the mesophyll with true perithecial walls, definite ostiola penetrating the epidermis, with many fine periphyses, and very fine branched paraphyses within the ascal cavities. The ascospores are very long and filiform. The perithecia are not in a continuous stroma, but within the epidermis there are enough blackened semi-coalesced hyphae to be called a clypeus. Therefore the genus *Ophiodothella* must be removed from the Dothideales and placed in the Sphaeriales.

The genus *Scolecodothis* Theiss. & Syd. (**18**: 277) was made on the species *hypophylla* (Theiss.) and according to the description is very closely related to *Ophiodothella,* if not identical. Shear and Clements (**16**: 92) placed both in the subfamily Phyllachorae of the Dothideales, and separate them only on: paraphyses present—*Scolecodothis,* paraphyses lacking—*Ophiodothella.* The writer finds that there are paraphyses in *Ophiodothella,* and in fact von Höhnel (**19**: 612) in the diagnosis says "paraphysen dunnfadig, sparlich," which would place these genera together according to Shear and Clements. Theissen and Sydow (**19**: 177) however, separate *Scolecodothis* and *Ophiodothella* on the fungus being between the epidermis and the palisade for the former, and lying in the mesophyll for the latter. If this character is constant then *Scolecodothis* should be in the same family with *Ophiodothella,* but not synonymous. The writer examined part of the type, but found only the conidial stage.

Another possible synonym for *Ophiodothella* is *Scolecodothopsis* Stevens (**17**: 183) based on the species *Ingae* Stevens, material of which has been loaned the writer by Stevens. The same generic characters as those of *Ophiodothella* were discovered here and the name has been reduced to synonomy. Shear and Clements (**16**: 293) so cite it.

Chardon (**15**: 60) describes the genus *Clypeotrabutia* with char-

acters similar to those of *Ophiodothella*. His type species is *portoricensis* (Stev.). In the generic description, he says spores one-celled and hyaline, which would include spores of any shape. In his description of the species, however, he says spores filiform, which would of necessity place his genus as a synonym under *Ophiodothella*. Then later, Chardon (**1**: 269) describes new species with eliptical spores. From mycological precedent, forms with filiform spores have generically been separated from those with other shapes, so such a species is not cogeneric with the type of *Clypeotrabutia*, and this genus is listed below as a synonym under *Ophiodothella*.

Shear and Clements (**16**: 259) cite *Clypeotrabutia* as a synonym under *Causalis* Theiss. Theissen (**20**: 184), in an illustration of the type species, *Myrtacearum* Theiss., shows perithecia in an enlarged stroma resting on the palisade and becoming erumpent through the epidermis, which is an entirely different situation from *Clypeotrabutia*, in which the perithecia are not stromatic and lie in the mesophyll.

The presence of two opposite ostiola in the perithecium of *Ophiodothella Vaccinii* does not seem to be sufficient grounds for the creation of a new genus. J. Müller, however, did erect the genus *Diachora* from a *Phyllachora* species which had opposite ostiola in the perithecia, but Theissen (**19**: 435) does not think that this is sufficient for a separation. Also, Shear and Clements (**16**: 293) refuse to recognize the genus *Diachora*.

DESCRIPTION OF THE ORGANISM

OPHIODOTHELLA Höhn., Frag. Myk. no. 630, 1910.

Scolecodothopsis Stevens, Ill. Biol. Monog. **8**: 183, 1923.

Clypeotrabutia Chardon, Sci. Surv. Porto Rico **8**: 60, 1926.

Ophiodothella Vaccinii sp. nov.

Type: no. *7091*—Univ. Georgia Crypt. Herb.—April, 1933, Athens, Georgia. Cotypes deposited in Nat. Herb., Washington, D. C.; Harvard Crypt. Herb.; Kew Herb.; The New York Botanical Garden.

Plate 51, text figures 1–2.

Maculae epiphyllae juvenes in foliis vivis 1–5 mm. diam. orbiculares non zonis cinctae margine aurantiaces; maturae in foliis deciduis vel mortuis

3–13 mm. diam. orbiculares plus minusve confluentes epidermide nigrescentae speciem stromatis facientae, mycelio fusco in utrisque stratis epidermidis pseudoclypeum formante; compluribus peritheciis in medio mesophyllo immersis 336 μ diam., 168 μ alt., duobus ostiolis, altero supra altero infra protrudente; ostiolo minute papillato puncticulato 70 μ diam., 98 μ alt., ex epidermide protrudente; parietibus perithecii 28 μ crassis ad aequatorem, 6–14 μ crassis ad aliam partem, investis ascis praeterquam in ostiolis; ascis ad centrum perithecii directis, cylindricis parietibus tenuibus, iodino +, subsessilibus 75.6–98 $\mu \times$ 11.2 μ, octosporis; ascosporis filiformibus guttulatis hyalinis curvatis cum ex asco liberatis, apicibus obtusis 67.2–97.4 $\mu \times$ 2.8 μ; paraphysibus multis filiformibus ramosis septatis, apicibus liberis.

Acervuli subepidermales in maculis laxe dispersi et elevate; strato conidio 358.4–476 μ diam., 84 μ crasso e strato parallelo conidiophororum oriundo; conidiis filiformibus plus minusve curvatis hyalinis continuis 28–58 $\mu \times$ 2 μ ab epidermide rupta extrusis.

In foliis *Vaccinii arborei,* Res publica coniuncta meridionis orientalis; peritheciis in Martio et Aprili maturantbus, conidiis in aestive et autumno.

Spots epiphyllous; young spots on living leaves, 1–5 mm. in diameter, orbicular, not concentric, orange-colored margin; mature spots on fallen or dead leaves, 3–13 mm. in diameter, orbicular, more or less confluent, epidermis becoming black giving appearance of stroma, dark mycelium in both layers of the epidermis forming a pseudoclypeus; several perithecia central in mesophyll, 336 μ in diam., 168 μ high, with two ostiola, one protruding through the upper epidermis, the other through the lower; ostilum minutely papillate, minutely punctate, 70 μ in diam., 98 μ high; perithecial wall 28 μ thick at the equator, 6–14 μ thick at other part, covered with asci except in ostiolar cavities; asci directed toward center of the perithecium, cylindrical, thin-walled, iodine reaction plus, subsessile, 75.6–98 $\mu \times$ 11.2 μ, eight-spored; ascospores filiform, guttulate, hyaline, curved when free from ascus, obtuse ends, 67.2–98.4 $\mu \times$ 2.8 μ; paraphyses many, filiform, branched, septate, free ends.

Acervuli subepidermal, scattered and raised in spots; conidial layer 358.4–476 μ in diam., 84 μ thick, arising from parallel layer of conidiophores; conidia filiform, curved, hyaline, continuous, 28–58 $\mu \times$ 2 μ, extruded through ruptured epidermis.

On *Vaccinium arboreum,* southeastern United States; perithecia maturing in March and April, conidia in summer and fall.

SUMMARY

1. The fungus on *Vaccinium arboreum* often determined in various herbaria as *Rhytisma Vaccinii* is a true pyrenomycete belonging to the genus *Ophiodothella.*

2. The developmental study of this form shows that it arises from a coil, the basal part of which is binucleate, the upper part forming a probable trichogyne of multinucleate cells. A definite wall forms, and there is a special feature in the two ostiola breaking out on opposite sides of the leaf. The ascal layer lies in an equatorial belt on the inner face of the perithecium which is different from the usual situation in the pyrenomycetes.

3. Members of this genus and related forms have been placed in the family Phyllachoraceae of the Dothideales, but the presence of the true perithecial wall, ostiolar formation, and paraphyses is indicative of their relationship in the *Sphaeriales,* and the genus *Ophiodothella* is here transferred to the family Clypeospaeriaceae of the Sphaeriales.

UNIVERSITY OF GEORGIA,
ATHENS, GEORGIA

EXPLANATION OF PLATES

PLATE 51

Ophiodothella Vaccinii. Fig. 1, Transverse section of the host leaf, showing a mature acervulus and feeding mycelium; 2, Portion of fig. 1 enlarged; 3, Conidiophore; 4, Conidium; 5, Longitudinal section of several coiled archicarps located in the middle of the host leaf, with trichogynes protruding through a stoma; 6, Longitudinal section of a young perithecium showing the oögonium and another cell of the coil in binucleate condition; 7, Longitudinal section of an older perithecium showing positions of branches from the oögonium and formation of the central cavity; 8, Longitudinal section of a perithecium showing the thickening of the equatorial parts of the wall and proliferation of the wall tissue at upper and lower extremities to form the two ostiola. Also, ascogenous hyphae are seen in an equatorial wall belt, intermingling with paraphyses.

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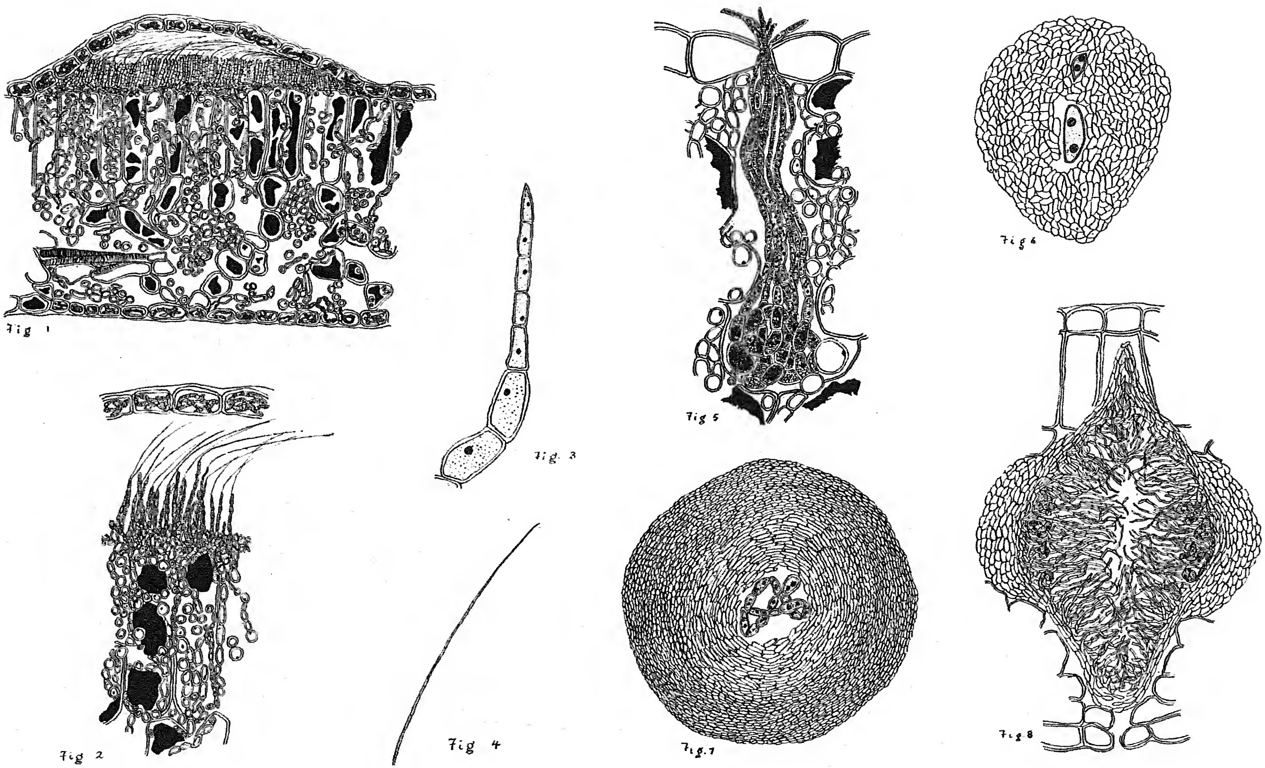


OPHIODOTHELLA VACCINII

NOTES AND BRIEF ARTICLES

The Myxomycetes

The volume under the above title by Thomas H. Macbride and G. W. Martin was issued on March 21, 1934, although the senior author quietly passed away a few days before the book was off the press. It is quite fitting, therefore, that an announcement of this new work and the portrait and brief biographical sketch of the senior author should appear in the same issue of Mycologia. The work is based on " North American Slime-Moulds " the second edition of which was published in 1922. To those familiar with this work scarcely more than an announcement is necessary.

The work has been completely revised and enlarged, consisting of 339 pages and 21 plates. Of necessity the enormous task of revising and completing this volume has fallen heavily upon the junior author and he should be highly commended for his efforts in putting through this task. The work is published by the Macmillan Company, as were the former volumes of North American Slime-Moulds.

Manual of the Rusts in United States and Canada [1]

Students of the plant rusts (Uredinales) have been anticipating for some years the appearance of Doctor Arthur's manual of this group upon which he has been working since the publication of " The Plant Rusts " (John Wiley & Sons, 1929). The reviewer believes that they will not be disappointed with the present volume.

Few groups of organisms present such difficult taxonomic problems as do the rusts. To a considerable extent this is due to their condition of obligative parasitism, regarding which our notions have materially changed since the passing of the last century. We have now come to recognize in part the effect of parasitism in producing morphological retrogression while at the same time physiological specialization is evolving many racial characteristics.

[1] The Science Press Printing Co., Lancaster, Pa., 1934. Price $6.00.

I consider the taxonomy of the rusts to have passed through five rather distinct periods. In the *first period* the basis of classification was strictly monomorphic, the importance of the life-cycle being unknown. Each spore-form was considered a distinct species.

The *second period* was initiated with the establishment of polymorphism by Tulasne and followed by the recognition of the interesting condition to which deBary gave the term heteroecism.

The *third period* came with the recognition of the relationship of the rusts with the Basidiomycetes and the acceptance of the teliospore as the significant stage in the life-cycle. As a result, Dietel's arrangement in Engler and Prantl's Pflanzenfamilien has been generally accepted.

In the meantime Schroeter (1889) introduced the biologic viewpoint, emphasizing the various cycles represented in the genus. This scheme enlarged upon by Arthur in 1906 as the basis of the taxonomic treatment presented in the North American Flora constitutes the *fourth period.* In this work Arthur stressed both biologic and morphologic characters, features which proved most stimulating to researches in the rust fungi.

Along with these developments has come the extensive studies in specialization by Eriksson, Klebahn, Stakman and others. These researches, together with Craigie's discovery of the function of the pycnium and the studies of Jackson and others on the origin of life cycles have served to emphasize the physiologic side of the problem and complicate very greatly the presentation of any natural system of classification.

Gradually the concept has arisen that the species consists of two stages of development (haploid and diploid). With the recognition of this fact and the knowledge that the morphology of the dominant spore form, the teliospore, is a fairly stable character, Dr. Arthur has brought together in this manual a classification which at least approaches what may be considered a natural arrangement.

With the appearance of this manual the *fifth period* is initiated. In this work Dr. Arthur has made a very definite attempt to bring together all species or forms of rusts which show degrees of relationship other than that indicated by hosts. This has necessi-

tated reducing some of the older recognized genera to sectional rank, as well as some recognized specific names to varietal rank. All this involves the nice question of species limitations. How distinct must a variation in life cycle, or in the more detailed morphological features become in order to be recognized as a generic or specific entity? The final answer has not been given but graduate students will surely find in this treatment of the rusts many problems which are stimulation to further researches.

The manual contains several unique aids for taxonomic work. A list of the names of authors of both rust species and host species is given together with their date of birth, and death dates in the case of those deceased. There is also an explanation of terms and usage, and a glossary which will be found helpful to the beginner.

Two families are recognized: Melampsoraceae with four tribes and fifteen genera, and Pucciniaceae with three tribes and seventeen genera. The descriptions are curtailed in comparison with those in the N. A. F. but sufficiently full to enable identification. Of great value are the illustrations prepared by Mr. George B. Cummins who has exhibited much skill and discrimination in presenting the outline drawings of the critical spore stages in nearly every species.

It is ardently hoped that this work will be only the beginning of a series of similar illustrated manuals of the different important groups of fungi.

C. R. ORTON

INTERPRETATION OF RULE 49BIS [1]

The fundamental purpose of the International Rules of Botanical Nomenclature is to secure a reasonable nomenclatural stability and uniformity of usage. The Rules were published and became effective in 1912 following the International Botanical Congress of 1905 and 1910. Rule 49*bis* applies solely to the Ascomycetes and Uredinales. What follows in this article pertains to the Uredinales, without in any way involving the Ascomycetes. The Rule

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station.

has not resulted in general acceptance, partly because of what are accounted inherent defects and partly from diversity of interpretation.

It is well in presenting a subject to clearly understand the significance of the terms employed. The Rule makes use of the terms "state," "stage" and "form," whose exact meaning is not necessarily apparent. The Rule, in so far as it relates to the Uredinales, reads thus:

"Art. 49*bis*. Among fungi with a pleomorphic life-cycle the different successive states of the same species (anamorphoses, status) can bear only one generic and specific name (binomial), that is, the earliest which has been given to the state containing the form which it has been agreed to call the perfect form, provided that the name is otherwise in conformity with the rules. The perfect state is that which ends in the teleutospore or its equivalent in the Uredinales. Generic and specific names given to other states have only a temporary value. They cannot replace a generic name already existing and applying to one or more species, any one of which contains the 'perfect form.'" [2]

To elucidate the Rule in dealing with the Uredinales it is well to remember that there are two *states* in every species of this order whatever its generic connection, the gametophytic or haploid, which bears aecidiospores, and the sporophytic or diploid, which bears uredospores and teleutospores. In the full life-cycle of a species there may be at times different *stages,* according to the kind of spores produced, as the aecidiosporic, uredosporic or teleutosporic stages. The body of spores evolved in any stage constitutes a *form.* In reduced species, especially the so-called short-cycle species, the two states are much curtailed, and some of the spore-forms may be suppressed, sometimes the aecidiospores, sometimes the uredospores, or in some species both.

The rule clearly sets forth that "the perfect state is that which ends in the teleutospore," or to agree with previous wording, "contains" the teleutospore. By inference the "perfect form" must be that of the sori bearing teleutospores. Neither the wording of the rule nor the history of spore development excludes the uredo-

[2] Briquet, John. Regles Internationales de la Nomenclature Botanique, 110 pp. June, 1912.

spore, a product of the sporophyte, and a normal part of the "perfect state," although the aecidiospore, a product of the gametophyte, is clearly excluded.

In applying the rule it should be borne in mind that the spores are not to be defined as to their structure, but only as to their origin or their mode of germination. Every permanent generic or specific name must be founded upon the "perfect state," which is equivalent to saying that it must be founded upon the sporophytic state, a state which bears uredospores and teleutospores. The test of a "form," in order to decide whether the name applied to it is to be considered a "permanent name" or only a "temporary name," must be either its origin or the germination of its spores or both, whatever the general appearance of the spores constituting the form may be.

If the definition of terms given at the beginning of this article be accepted, which the wording of the Rule logically requires, making the uredospore a product of the "perfect state . . . which ends in the teleutospore," the names applied to the uredosporic stage are not "temporary," but permanent, that is, acceptable, the same as those applied to the teleutosporic stage.

A common interpretation, however, makes the Rule exclude names from recognition in priority when applied to the uredosporic stage. This interpretation is not based upon the practice of uredinologists present or past, as a statistical study clearly shows, but can be traced to the impression held by many botanists, both mycologists and others, that as uredospores usually (but not always) function for the dispersal of the species they are of the nature of conidia,[3] and therefore should not be considered as belonging to the "perfect state."

Uredospores, however, are borne on sporophytic mycelium and are binucleate, a condition which is not generally true of conidia as ordinarily considered. The same sorus producing uredospores often produces teleutospores, either at the same time or later. If a species does not produce uredospores in the cycle of development, the teleutosporic sori may wholly or in part (*e.g.*, in *Puccinia veronicarum*) become pulverulent, and scatter their spores in the same manner and quite as effectively as any uredospores. True

[3] Gwynne-Vaughan, Fungi, 1922, p. 4.

conidia, as in the Ascomycetes and higher Basidiomycetes, are borne on the gametophytic mycelium and are haploid. Such spores do not occur in the Uredinales. The adaptation for rapid dissemination is not inherent in any one kind of spore. The fact that the uredospores play the rôle of rapid propagation does not entitle them to be called conidia.[4]

Not only is the assumption incorrect that uredospores are of the nature of conidia, but usage as shown by the publications of systematic uredinologists is opposed to such an interpretation of the Rule. The only recent work embracing all known species of the Uredinales from every part of the world is that of Sydows' Monographia Uredinearum. The work is accurately compiled, and the synonymy is especially complete. The names of species recognized are with rare exceptions those which are in general use.

It is interesting to note that out of 2333 species embraced in this work having teleutospores only 26 species are affected by the varying opinion regarding the uredospore, and that in every instance to exclude from priority the names applied to the uredosporic stage of these 26 species will require a change from the well established and generally accepted names of long standing and the substitution in most instances of unfamiliar names. Moreover, to invalidate uredosporic names would permit the changing of the specific names of more than 400 species now known only under the genus *Uredo,* when their teleutospores are discovered, a number to which many names are constantly being added.

It may be asked why make a change for so small a number as 26 names out of 2333 species; and as to the possible substitution of new specific names under the genus *Uredo* upon the discovery of teleutospores it may be asserted that no serious mycologist would intentionally do it. In answer, let it be distinctly understood that no change in the Rule is advocated. The Rule is to stand as it is. What is needed is a better understanding of the individual development of the rusts, and a clearer interpretation of the Rule. By so doing and accepting the Rule as it stands the 26 species referred to will have the specific names now in general use validated, no changes being required, and the specific names of 400 or more

[4] Gäumann, Vergleichende Morphologie der Pilze, 1926, p. 435; English translation by Dodge, 1928, p. 568.

species now only known in the genus *Uredo* made permanently acceptable. It is only by insisting that uredosporic names should not be recognized in priority that changes for the 26 names are required, and which permits changes for names now under the genus *Uredo*.

Following Rule 49*bis* is a paragraph giving "Examples." These pertain to the genera *Aecidium* Pers., *Caeoma* Link, and *Uredo* Pers., as they are used to designate different stages in rust development. It is not made clear that these three names have at no time been abandoned as generic names. They were first applied to stages by de Bary in 1863, who authorized at the same time the use of the terms aecidiospore and uredospore and also the wholly new term teleutospore. An error is made in stating that *Melampsora* (1848) is antedated by the use of *Uredo* to designate a state (1863). The names *Aecidium, Caeoma, Uredo* and some others, have by general consent long passed into synonmy as standard generic names, although for convenience they are retained as "form-genera." The paragraph following the Rule should be reworded to remove the confusion resulting from the use of names both for stages and for genera. *Caeoma* Link is a synonym of *Phragmidium*, and the names *Aecidium* and *Uredo* need to be placed in the list of *genera conservanda* as synonyms of *Gymnosporangium* and *Melampsora* respectively.

It may be well to call attention in this connection to the fact that a specific name is not invalidated by being first published under a wrong genus, even one not belonging to the Uredinales. Many early names were established under such non-rust genera as *Ascobolus, Lycoperdon, Tremella, Oidium, Sphaeria*, etc., to which might be added the sometimes misplaced genera *Aecidium, Uredo* and others.

Theoretically the name first applied to a rust in any stage of its development, whether of gametophytic or sporophytic origin, should be considered the accepted name of the species, as the writer exemplified in the 7th volume of the North American Flora, but in practice and to maintain stability in the nomenclature it has been found desirable to exclude aecidiosporic names, or any others applied to the gametophytic (sexual) state, from recognition in de-

ciding priority. This is now acceptable to nearly or quite all uredinologists and is in accordance with the intent of the Rule.

Although having no direct bearing upon the nomenclatural problem, it is interesting to note that it is the uredo-stage that supplies the common name " rust," and its equivalent in ancient times, by which this group of fungi is best known to the non-technical public. The uredo-stage has always been recognized as a characteristic feature of the Uredinales. In fact, no other fungi possess spores of like nature, although sometimes possessing spores having a superficial resemblance and erroneously designated as species of *Uredo*. It was the uredosporic stage of the rusts that suggested to Persoon in 1818 the name Urédinées for the group, which later became Uredineae, and finally Uredinales.

Conclusion

Rule 49*bis* of the International Rules of Nomenclature is written in involved phraseology, but when fully understood supports the general practice of systematic uredinologists, both past and present. The Rule excludes aecidiosporic names (of the gametophytic state), but includes uredosporic and teleutosporic names (of the sporophytic, or " perfect " state). This interpretation accords with the individual development of the rusts, and also promotes stability and uniformity of usage, which are the basic purposes in promulgating the Rules.

The examples cited, following Rule 49*bis*, are confusing, and not wholly accurate. They need to be rewritten.

Incidentally it may be pointed out that the uredosporic stage is the most characteristic feature of the Uredinales, and the one to receive popular recognition. Moreover, it is the stage which gives the only name by which the order has at any time been known.

J. C. ARTHUR

TWENTY-FOUR YEAR INDEX

The proposed Twenty-four Year Index of MYCOLOGIA is now in the process of printing and it is expected that this will be issued early in 1935. It is estimated that it will comprise approximately 275 pages. The index will consist of two parts:

1. An alphabetical list of authors with full titles of their papers arranged chronologically.

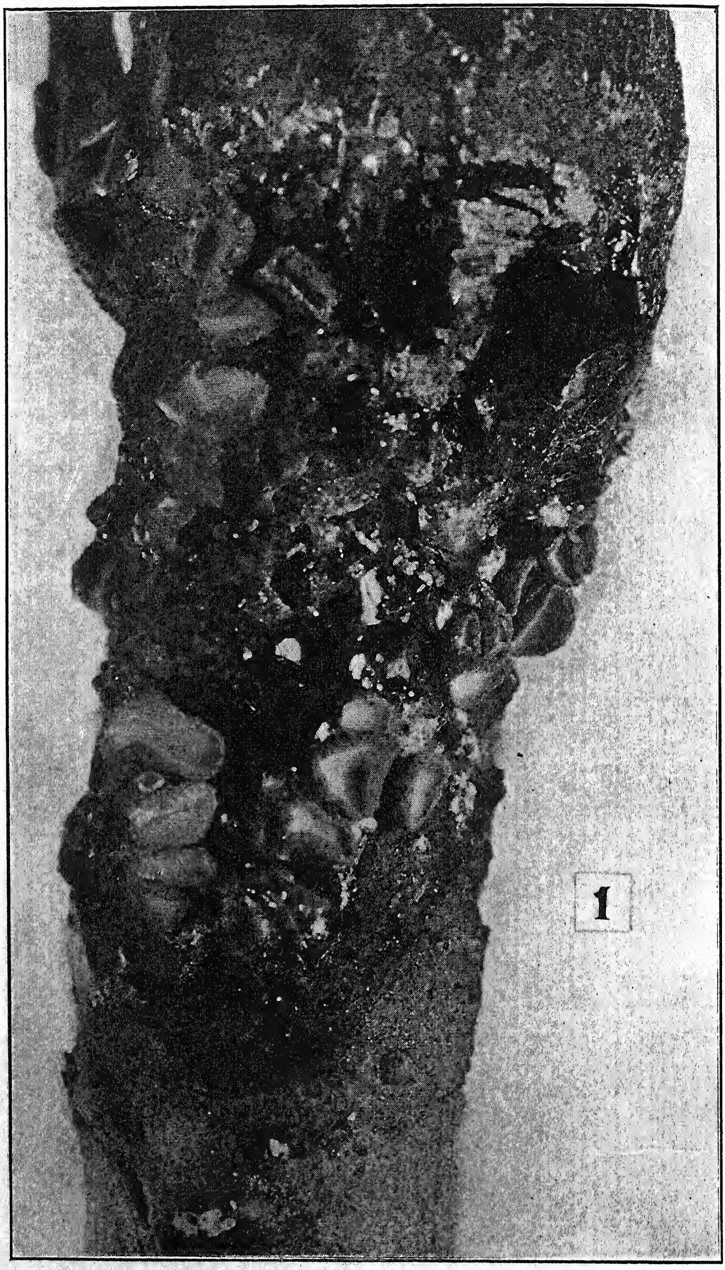
2. An index to genera and species and their subdivisions of the fungi including myxomycetes, bacteria, and lichens.

This index will be issued in two forms, in paper covers or in board covers with fabrikoid. The pre-publication price is $2.50 for the former and $3.00 for the latter. Any orders entered before December 31, 1934 will be filled at this rate. After January 1, 1935 the price will be increased $.50 on each. In placing orders be sure and indicate whether you wish your copy in paper covers or in board covers bound in fabrikoid.

OBITUARY

Dr. Karl Frederic Kellerman, for many years Associate Chief of the Bureau of Plant Industry, died August 30, at Washington, D. C. Dr. Kellerman, in addition to his administrative duties, directed the campaign for the eradication of Citrus canker and the phoney disease of peach. He was instrumental in establishing the Journal of Agricultural Research and served 11 years as chairman of its editorial board. He was appointed to the National Research Council in 1917 by President Wilson and later became a member of the Division of Biology and Agriculture. He was for a number of years a member of the Federal Horticultural Board and took an active part in furthering the work in plant quarantine carried on by this organization. Dr. Kellerman became Chief of the Division of Plant Disease Eradication in the Bureau of Entomology and Plant Quarantine in 1933. It will be remembered that he was the son of the late Dr. W. A. Kellerman, one of the founders of the Journal of Mycology and a mycologist of note.

JOHN A. STEVENSON



Dasyscypha Pini

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DASYSCYPHAE ON CONIFERS IN NORTH AMERICA. III. DASYSCYPHA PINI

GLENN GARDNER HAHN AND THEODORE T. AYERS

(WITH PLATES 52 AND 53)

INTRODUCTION

In the first two papers of this series the authors discussed the taxonomy of the European larch canker parasite, *Dasyscypha Willkommii* (Hartig) Rehm (**4**), introduced into the United States upon imported larch, and the native fungus, *D. Ellisiana* (Rehm) Sacc. (**5**), a commonly occurring native saprophyte upon species of pine and other conifers. The latter fungus has become parasitic upon introduced Douglas fir and pine species in New England and elsewhere along the Atlantic seaboard. The present paper is concerned with a very interesting highland Discomycete with brown exciple, *parasitic* upon five-needled and Scotch pines, occurring at high altitudes and in north latitudes in North America and Scandinavia. This parasite heretofore has been regarded generally as synonymous with the *saprophytic*, brown-excipled species, *Dasyscypha fuscosanguinea* Rehm (**13**), of central Europe. Certain mycologists have been inclined to question the identity of the parasitic organism with Rehm's fungus growing on pines in the Alps. Morphological differences had been noted between the two, but these were not considered sufficient to warrant specific rank for the former organism (**15**).

Survey work in the Lake States and in the Pacific Northwest,

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both in the United States and Canada, which has been concerned with the distribution and spread of white pine blister rust in the West, produced some very interesting collections of a conspicuous pine canker, somewhat similar to that caused by white pine blister rust (**6**, **15**) and very much like that caused by *Dasyscypha Willkommii,* upon native five-needled pines—*Pinus Strobus* L., *P. monticola* D. Don and *P. albicaulis* Engel. Invariably these resinous lesions were closely associated with apothecia, covered with brownish hairs, whose discs were a brilliant orange-yellow. Because of their exceptional size and beauty they made the malignant lesions upon which they fruited abundantly, very striking, particularly in damp weather.

The morphological characters of this North American *Dasyscypha* parasite, associated with a destructive pine canker, were very much like those given by Brunchorst (**1**), Lagerberg (**11**), and Jørstad (**7**) for a Scandinavian fungus causing a similar canker in the Arctic Circle regions. The investigation has shown that the North American parasite is practically identical with the Scandinavian *Dasyscypha* form on *Pinus sylvestris* L. Morphological as well as physiological data are given substantiating the specific rank accorded this highland pathogen of northern latitudes in Europe and in America.

HISTORY

Lagerberg (**11**), in a comprehensive paper discussing a canker and parasite of *Pinus sylvestris* growing in the pine barrens of northern latitudes in Scandinavia, pointed out that Brunchorst (**1**, p. 9) was the first author to draw attention to the parasitic nature of a brown-excipled *Dasyscypha.* Lagerberg called the pathogen *D. fuscosanguinea.*

Previously in 1892 the Norwegian mycologist had described the parasite as *Lachnella Pini* n. sp. and had given not only an excellent description of the fungus but also an adequate account of the disease (**1**, FIG. 3; PL. without number or legend). Brunchorst's name for the highland parasite would probably have been recognized by Lagerberg and other pathologists and mycologists but for the fact that Karsten (**10**) had maintained that the pine pathogen in Norway was synonymous with Rehm's saprophytic

species, *Dasyscypha fuscosanguinea* (**12**, **13**), from the Tyrolese Alps. Apparently there has been a general acceptance of Karsten's opinion and the organism described by Brunchorst has been regarded as identical with Rehm's brown, hairy-cup fungus.

In 1881, when Rehm first described *Dasyscypha fuscosanguinea* (**12**), which he later characterized as a "Hochalpenbewohner" (**13**), he considered his fungus as occurring saprophytically on dead branches of *Pinus Cembra* L. and *P. pumilio* Haenke. It is notable that in his seond description (**13**, p. 848) where a synonomy is given, Brunchorst's *Lachnella Pini* is not listed, but in the addenda at the end of the volume (**13**, p. 1268) he cited Brunchorst's fungus as synonymous with his species, following Karsten. In his discussion Rehm (**13**, p. 849) questioned Karsten's new combination *Lachnella fuscosanguinea* (Rehm) for a Finnish fungus on dead branches of *Pinus sylvestris*. There was reason for this doubt because Karsten (**9**) apparently was concerned with another fungus—a large-spored, white-excipled form related to Hartig's *Dasyscypha* (*Peziza*) *Willkommii*. Karsten (**10**) apparently receded from his former judgment, for in 1895 he reverted to Rehm's usage of *Dasyscypha* for the Tyrolese species. In view of Karsten's confused thinking with regard to the identity of both Rehm's and Brunchorst's species, it is unfortunate that his opinion should have had weight.

Among Rehm's personal herbarium specimens of *Dasyscypha fuscosanguinea*, now preserved at the Botanical Museum, Stockholm, is included the pine parasite collected by Lagerberg (**11**) in 1911 in northern Sweden, 65° n. lat., and determined by him as *D. fuscosanguinea*. Rehm had distributed this specimen as Ascomyceten *112c*. *Dasyscypha fuscosanguinea* Rehm, without annotation or particular comment. The inclusion of this fungus, which apparently Lagerberg regarded as identical with Brunchorst's parasite, among specimens determined by Rehm as his own species, came after that German author had published his final description. Apparently Rehm himself was not cognizant of morphological differences or the parasitic nature of the Arctic fungus. Since the time of Rehm, the northern European parasite has been accepted as synonymous with *D. fuscosanguinea* even in

Scandinavia where the organism has been known for a long time and where it has been studied most.

Recently in 1929, Stillinger, reported a *Dasyscypha* species from North America with brownish excipular hairs and orange-yellow disc as *D. fuscosanguinea* (15), which he described as a parasite of western white pine (*Pinus monticola*). This fungus was probably first collected in August, 1922, along the western edge of Glacier National Park in Montana. In 1925, Dr. J. S. Boyce, who had become interested in the pathogen, sent specimens to Dearness for determination. The Canadian mycologist, who was the first to publish on the organism in this country, recognized a great similarity between the Idaho parasite and the so-called *D. fuscosanguinea* of Lagerberg from northern Sweden, but he was inclined to question the identity of the parasite with Rehm's saprophyte occurring in the Tirol. Recognizing morphological as well as physiological differences, he (2) commented as follows upon northern Idaho material, parasitic on *P. monticola*, collected in 1924 by Boyce: "(?) *Dasyscypha fuscosanguinea* Rehm. . . . This compared with material collected by Lagerberg on *P. silvestris* in Sweden, labeled as above and passed upon by Rehm, is paler externally, more luteous on the disc and has longer paraphyses, *but on the whole it is very similar*. It is hardly sanguineous and if it is the same species as the European one it must be much more destructive by inference from Rehm's statement of habitat, 'an durren Aestchen'—on dry branchlets."

When Stillinger (15) published his interesting paper on *Dasyscypha fuscosanguinea*, he expressed the opinion, concurred in independently by J. R. Weir, W. W. Diehl and Dearness, that the North American fungus could very properly be recognized as the parasitic form of *D. fuscosanguinea*. Stillinger cited differences in sizes of asci and spores between the American parasite and Rehm's fungus as given in the original description. Apparently a comparison was not made with Lagerberg's parasitic material that Dearness (2) had examined and reported upon.

The authors were greatly impressed by the fact that specimens of the highland parasite were always to be found in direct association with pronounced cankers. This fungus apparently did not occur saprophytically as did *D. fuscosanguinea*. The life

habits of these two organisms were similar to those previously discussed (4) for the parasite, *D. Willkommii* and the nearly related, but in this case, closely associated saprophyte, *D. calycina* Fuckel (nec *Peziza calycina* Schum. and *P. calycina* Fries). An examination of his personal specimens of *D. fuscosanguinea,* which had been carefully annotated by Rehm, and a comparison of these with herbarium and fresh European and North American material of the highland parasite, convinced the writers of the separate identity of the two organisms.

EXAMINATION OF DASYSCYPHA FUSCOSANGUINEA SPECIMENS IN REHM'S PERSONAL HERBARIUM

To those unaware of Rehm's method of recording observations on the Discomycetes which he reported, it may be of interest here to note that the great German mycologist carefully recorded the measurements of spores, asci, paraphyses and hairs with supplementary descriptive detail in fine German script, upon small pieces of note paper attached to the specimen packet. Upon bits of coördinate paper pasted to these, he delineated the typical shape and size of spores in the material studied. Most of the *D. fuscosanguinea* specimens were so annotated. Accordingly we were able to examine probably the actual specimens Rehm had studied when he wrote the descriptions for his species.

A study of his personal collections of *Dasyscypha fuscosanguinea* showed that at least two morphologically distinct groups of brown-excipled forms were involved, one, saprophytic on cones and dead twigs and branches of *Pinus pumilio, P. Cembra, P. montana* Mill. and *P. Mugho* Poir., having shorter spores and asci than those of a second group, represented by a single unannotated specimen collected in northern Sweden on *Pinus sylvestris* by Lagerberg. *The fungus collected by Lagerberg was the only one indicating a parasitic habit by the production of a canker.* The packet enclosing this specimen contained the Swedish label: "Flora Suecica. *Dasyscypha fuscosanguinea* Rehm. Prov. Vasterbotten, Staatforst Östra Jörnsmarken, Juli, 1911. Kiefernheide, 65° n. Breite, legit. T. Lagerberg." It should be noted that Rehm in distributing this collection as Ascomceten *112c D. fuscosanguinea* Rehm, had previously assigned this number to *"112c Dasyscypha*

fuscosanguinea Rehm var. *aurantiaca* v. Höhnel," collected in 1904 by von Höhnel, which he (Rehm), according to his herbarium notes, apparently regarded as synonymous with his own species.

Because of the evident morphologic differences alluded to in the foregoing, between the saphophytic and parasitic organisms, the authors are of the opinion that these two fungi are distinct species and have given the following amended descriptions of *Dasyscypha fuscosanguinea* and the Scandinavian pathogen, of Scotch pine. Although they have not seen Brunchorst's original specimen, a study of Scandinavian material which showed agreement with his description of the type and the canker disease associated with the organism (**1**), assured the authors that they very probably were concerned with Brunchorst's species. Accordingly they have reaffirmed the specific identity of his fungus and have made a new combination (listed below) to designate the highland parasite. Morphological observations, which agreed with those made by Lagerberg (**11**), Dearness (**2**) and Stillinger (**15**), indicated differences in color, in the size of spores, asci and paraphyses, and in the shape of the hairs and paraphyses.

DESCRIPTIONS OF THE LARGE-SPORED, BROWN-EXCIPLED DASYSCYPHAE

DASYSCYPHA FUSCOSANGUINEA Rehm, desc. emend.; Ascom. *112* nomen nudum 1872; Ber. Nat. Ver. Augsburg **26**: 30. 1881; Sacc. Syll. Fung. **8**: 459–460. 1889; Rabenh. Krypt.-Fl. **1**^{3}: 848–849. 1896.

nec. *Lachnella fuscosanguinea* (Rehm) Karst. (Symb. Myc. Fenn. **24**: 16. 1888); *L. Pini* Brunchorst (Karsten, Hedwigia **34**: 8. 1895).

Syn: *D. fuscosanguinea* var. *aurantiaca* v. Höhn. apud Rehm (Ann. Myc. **3**: 230. 1905); (?) *Trichopeziza fuscosanguinea* Lambotte (Myc. Belg. **1**: 294. 1887).[1]

Apothecia separate or aggregate, sparse or moderately abundant, waxy, leathery, distinctly stalked, at first globose, urn-shaped, ex-

[1] Rehm (**13**) was inclined to doubt that *Lachnella confusa* Karst., on Scotch pine from Finland, listed in the synonymy of *Dasyscypha fuscosan-*

panding as a flat disc, hymenium "blood-red" to "reddish-yellow" (12, 13) when dry; exciple (dry) covered with Buckthorn brown, Dresden brown to cinnamon-brown [2] hairs ("dark brown" according to Rehm); cups 2–6 mm. diam., commonly 2–4 mm. diam., stalk about 1 mm. long; when dry the exciple folds lengthwise against itself, concealing the disc which with age becomes ochraceous, or in large apothecia, the folding may occur from three places on the margin; hairs, simple, elongate, septate with short cells, exceedingly scabrous with minute roughenings, microscopically brownish-olive, cylindrical, broadly tapering in some, with blunt apices, obtusely rounded or subacute, 3–4 μ wide; asci cylindric, clavate, extreme range, (40) 70.0–90.8 $\times$ 7.2–10.6 μ; ascospores, 8 in ascus, uniseriate or biseriate, hyaline, straight, unicellular, becoming uniseptate upon germination, ovate, elliptic or elliptic-ovate with blunt, obtusely rounded extremities, extreme range, (180) 10.6–16.0 $\times$ 4.0–6.2 μ, commonly, 12–14 $\times$ 4–5 μ; paraphyses filamentous, filiform, outranking asci, septate, with yellowish oil drops, 1.5–2 μ broad, unswollen at the apex or very slightly swollen above, apices 3 μ broad.

Imperfect stage not observed. This species has not been grown in pure culture and it may as in the case of *D. arida* (Phill.) Sacc., which has been found to produce the imperfect stage readily in culture, do likewise.

Hab. A saprophytic highland fungus of the Tirol. Occurring on bark of dead branches: *Pinus Cembra,* Kühtei, (Oetz) Tirol (c. 6200′), coll. Rehm; *P. pumilio,* Arlberg, (c. 5900′), and Peischlkopf, Tirol, (c. 5900′), coll. Britzelmayr; *P. montana,* Rudolfshütte, Stubachthal (Salzburg), coll. von Höhnel. On cone scales of *P. pumilio,* Algäu, Alps, coll. K. Arnold, and *P. Mugho.*

Dasyscypha fuscosanguinea is not known to occur in Scandinavia. Whether it occurs in North America is problematical. It

guinea was identical with his own species. We have examined the "Originalexemplar" of *L. confusa* alluded to by Rehm in his discussion (13, p. 849). The specimen now consists of merely a slender Scotch pine twig entirely lacking in apothecia. Accordingly we are not in position to give the characteristics of the fungus, and only can state that a canker was not present in the specimen.

[2] The color nomenclature is that of R. Ridgway, Color standards and color nomenclature. Washington, D. C. 1912.

is distinct from the shorter-spored, brown-excipled, saprophytic species, *D. arida,* first collected by Harkness on pine bark in the Sierra Nevada Mts., California, in 1876 (Grevillea **5**: 117, PL. 89, FIG. 13. 1877) and widely distributed in western North America. Whether *D. flavovirens* Bres. (Fungi Tridentini **1**: 92, PL. 104, FIG. 1. 1887) collected on *Larix europaea* in the Alps (Ortler, Eifischthal and Kals) is distinct from *D. fuscosanguinea* will be discussed in a later paper of this series.

Exsiccata examined from Rehm's personal herbarium:

"*112. Dasyscypha fuscosanguinea,* mihi nov. spec. ad interim. An abgehauenen Aesten von *Pinus Cembra* bei Kühtei (Oetz) in Tyrol c. 6200′ 8/1872. Dr. Rehm." Rehm recorded ovate-elliptic spores observed in this specimen as being 12–15 × 4–5 μ; asci, 70 × 9–10 μ; paraphyses filamentous and swollen at the tip. A measurement of spores from this collection gave the following extreme range, (100) 10–16 × 4–6 μ, commonly 12–14 × 4–5 μ; asci (10) 68.6–79.4 × 8.0–10.6 μ. Although scant in number, the ascocarps, which occurred saprophytically, were in excellent condition.

"*112b. Dasyscypha fuscosanguinea* Rehm. Auf dürren Aesten von *Pinus pumilio* über dem Maiensee auf dem Arlberg in Tyrol c. 1800 m. 8/1879. Britzelmayr." Rehm annotated this specimen: ovate spores, 12–14 × 4–5 μ; "1½ reihig"; asci, 75 × 9 μ. A slide made from the identical specimen examined by Rehm (indicated by a bit of paper) showed spores with extreme range, (20) 11.0–14.2 × 4.2–6.2 μ; asci, (10) 72.2–91.6 × 7.2–10.4 μ.

"*112c. Dasyscypha fuscosanguinea* Rehm var. *aurantiaca* v. Höhn. An Rinde von *Pinus montana.* Rudolfshütte im Stubachthal (Salzburg) 8/1904. Dr. v. Höhnel." Rehm annotated this specimen: "= Rehm Discomyc"; spores, elliptic-ovate, 10–12 × 4–5 μ; asci, 60–100 μ. This is a particularly fine specimen, splendidly preserved and showing abundant, large ascocarps occurring saprophytically. Measurements of spores, extreme range, (20) 11.0–15.2 × 4.0–5.4 μ; asci, (10) 80–88 × 8–10 μ.

The following specimens, which were all found to be in agreement morphologically with the foregoing material, were also ex-

amined and slides made for study when ascocarps were available:

D. fuscosanguinea Rehm. "mikroscopisch = Rehm. Ascom. *112.* An *Pinus pumilio,* Peischlkopf in Tyrol, c. 1800 m., July, 1878. Britzelmayr."

D. fuscosanguinea Rehm. *Pinus pumilio,* Tyrol, June, 1884. Collected and identified by Rehm, and annotated by him: spores elliptical, 12–14 × 4 μ, in 2 rows; asci, 60 × 8 μ; paraphyses filamentous; hairs c. 4 μ.

D. fuscosanguinea Rehm. Cone scales of *Pinus pumilio,* 1909, K. Arnold. Ascocarps 1–2 mm. diam. were very abundant in the specimen; spores, extreme range, (10) 11.2–15.8 × 4.4–5.6 μ.

No. 19. *D. fuscosanguinea* Rehm. Cone scales of *Pinus pumilio.* Algäu 8/1909, K. Arnold. Rehm annotated this specimen: spores ovate-elliptic, 12 × 4 μ; in 2 rows; asci, 70 × 9 μ; paraphyses, filamentous 1.5 μ. Spores showed the following extreme range, (10) 12.8–15.4 × 4.8–5.6 μ.

No. 28. *D. fuscosanguinea* Rehm. Cone scales of *Pinus Mugho,* July 3, 1910. Annotations by Rehm: ovate, elliptic spores, 12 × 4–5 μ; in one row; asci, 70 × 9 μ. The ascocarps had completely disappeared in this specimen.

No. 4. *D. fuscosanguinea* Rehm on *Pinus montana,* c. 1100, m., July 3, 1910. Rehm annotated this specimen: elliptic spores, unicellular, finally 2-celled, 12 × 4 μ; in one row; asci, 80 × 8 μ; paraphyses, filamentous, 2 μ, above 3 μ. No ascocarps in this collection.

The description which follows is that of the highland parasite originally described by Brunchorst (1). Lagerberg (11) adequately figured the fungus and discussed at length its parasitic relationship and the canker lesions associated with the fungus.

Dasyscypha Pini (Brunchorst) comb. nov., Descr. emend.

Syn: *Lachnella Pini* Brunch. Bergens Mus. Aarbog. no. 8: 8–11. 1892.

Dasyscypha fuscosanguinea Rehm, pro parte (sec Karsten: Hedwigia **34**: 8. 1895; Rabenh. Krypt.-Fl. **1**^{3}: 1268. 1896).

D. monticola Diehl, in herb., Division Mycology, B. P. I., U. S. D. A.

Apothecia separate or aggregate, occurring abundantly on resinous cankers (1, PL. without legend; **11**, p. 146, FIG. 9) similar to those formed by the European larch canker parasite, *D. Willkommii* (Hartig) Rehm. Since the fruit bodies are often developed in large numbers and are closely crowded together, they present a beautiful sight because of their splendid color and large size (PLATE 52, FIG. 1), when the cups are fully expanded in moist weather. Apothecia distinctly stalked even at an early age, at first globose, urn-shaped, expanding as a flat disc with a bright orange hymenium, surrounded by marginal hairs covering the exciple, macroscopically decidedly pale cinnamon in color; 2–4 mm., occasionally 5 mm. diam.; with age the disc becomes a yellow-ochre, and the pale hairs may darken to a cinnamon-brown; when dry the exciple folds against itself over the center in an elongate fashion concealing the disc, and in large apothecia, the folding may occur from three places on the margin; hairs, simple, elongate, septate with short cells, moderately scabrous with minute roughenings, microscopically pale olive-buff, concolorous, longest hairs filiform, tapering, attenuate with exceedingly slender acuminate apices, 1–2 μ broad, extremities of shorter cylindrical hairs, subacute or obtusely rounded, 3–4 μ wide; extremities of asci cylindric, clavate, apex obtuse, extreme range, (70) 88.4–123.4 $\times$ 7.2–11.8 μ; ascospores 8 in ascus, uniseriate, hyaline, straight, unicellular, commonly becoming bicellular on germination, two or three septa, however, may be laid down, elongate elliptical, occasionally pyriform, with one apex obtuse, the other tapering to a sub-acute or acute extremity, extreme range, (272) 13.0–22.0 $\times$ 4.4–7.0 μ, commonly 15–20 $\times$ 5–6 μ; paraphyses, filamentous, outranking asci, septate, with yellowish oil drops, branched at base, of equal diameter, 1.5–2 μ wide, unswollen above (**11**, p. 147, FIG. 10).

Imperfect stage not observed in nature. Numerous mono-ascus and -spore cultures of North American and Scandinavian material on synthetic or natural pine twig media, did not produce the imperfect stage either at room temperature or in the refrigerator at lower temperatures (38°–42° F.).

Hab. The species is restricted to the genus *Pinus* occurring as a parasite in resinous cankers. It is reported on living *P. sylvestris* by Brunchorst from northern Norway—Finnmark (Karasjok,

Alta), Ranen (Mo) and Valley of Målselve, 66°–69° n. lat.; by Jørstad from Norway farther south—Trondheims Bymark, 63° 27′ n. lat. and Telemark, Fyresdal, 59° 10′ n. lat.; by Lagerberg from northern Sweden—Luleå distrikt (Tärendö and Gällivare), Province Vasterbotten, Staatforst, Östra Jörnsmarken (Umeå distrikt) and in the most southern region, Gäfle-Dala distrikt (Särna in Dalarno), 61° 30′–67° n. lat.

Karsten (9) reported the fungus from Finland on Scotch pine but from his description it is extremely doubtful if he was concerned with the same fungus described by Brunchorst.

With regard to the distribution of the fungus, Jørstad (7) comments on it as follows: "Since the time of Brunchorst this pine disease has not been studied much further in Norway, and we have, consequently, no new data. I have seen no material of this fungus from southern Norway, but I feel sure it will appear at least in the more elevated pine-wood. It seems to be a pronounced parasite, even if it may live also as a saprophyte and it seems confined to species of *Pinus*" (translation by Theo. Holm). As noted above Jørstad (8) later found the fungus in Telemark which is the southernmost collection for the species in Scandinavia. It is interesting here to note that in Lind's revision of Rostrup's Danish Fungi (Copenhagen, 1913), the species is not listed.

In North America the fungus has never been found on Scotch pine but only on native species of five-needled pines. It has been reported by Stillinger from the West in northeastern Washington, northern Idaho, western Montana and British Columbia (Nelson) on *P. monticola,* at elevations of from 3000′–6000′. According to observational records in the herbarium of Dr. Boyce, the parasite which has not been found in the Cascade Mountains to the coast, has a frequent elevation of 3000′ or more, and is not found lower in the valleys.

The following western collections on native species of pine have been examined:

Pinus albicaulis. British Columbia:[3] *40521,* Mt. Revelstoke Nat'l Park, 4000′–5000′, 50° 45′ n. lat., coll. J. R. Hansbrough & A. A. McCready, Aug. 24, 1930 (PLATE 53, FIG. 1); *40522,* Flat

[3] Unless otherwise indicated, collection numbers denote specimens for study filed in the Division of Forest Pathology, New Haven, Conn.

Creek, 30 miles east of Revelstoke, 6000′–7500′, 51° n. lat., coll. J. R. H., Aug. 31, 1930; *40714,* D'Arcy, 3 miles west, 6000′, coll. J. R. H. & J. L. Mielke, June 1, 1931.

P. monticola. British Columbia: *53993* (Herb. P. S.), Mile *72* (Pacific Great Eastern RR. Station), Prohibition Claim Trail, Upper Birkenhead River Watershed, 4700′, coll. C. N. Partington & H. G. Lachmund, Sept. 1, 1927; *40731,* Revelstoke (10 miles north), 3500′, coll. J. R. H., Aug. 9, 1931. Washington: *2599* (Herb. C. R. Stillinger, dupl. in Herb. P. Spaulding), Mt. Spokane, 5700′, coll. H. N. Putnam, May 30, 1926; *41513,* Metalline Falls, Pend Oreille Co., 4500′, 48° 45′ n. lat., coll. H. N. P., W. A. Rockie & A. F. Lackey (dupl. *1811,* Herb. J. S. Boyce), Aug. 12, 1923. Idaho: *1208* (Herb. J. S. B.), Stony Creek Lookout, 30 miles northeast Elk River, Clearwater Co., 4500′–5000′, coll. C. R. S., Aug. 10, 1923 (dupl. in Myc. Coll., Bureau Plant Industry, Washington, D. C.); *2554* (Herb. C. R. S., in Herb. P. S.; dupl. *1208,* Herb. J. S. B.); *1452* (Herb. J. S. B.), Upper Priest Lake, Boundary Co., 3600′, coll. J. S. B., July 31, 1924; *2600* (Herb. C. R. S., dupl. in Herb. P. S.), Bear Skull, St. Joe National Forest, 6000′, coll. P. Rowe, Sept., 1927. Montana: *381* (Herb. School of Forestry, Univ. Idaho), Belton, west edge Glacier Nat'l Park, coll. C. R. S., Aug. 26, 1922.

P. Strobus. Michigan: *53083,* Keweenaw Co., Upper Peninsula, coll. J. K. Kroeber, Oct., 1930 (PLATE 53, FIG. 2); *53093,* Ojibway to Delaware Mine, Keweenaw Co., coll. L. W. Hodgkins, Oct., 1930, 47° 16′ n. lat.; *53149,* Michigame, Marquette Co., coll. L. W. H., Nov., 1930, 46° 30′ n. lat.; *53994,* 20 miles north of Calumet, Keweenaw Co., 1200′–1500′, coll. J. K. K., Sept., 1933.

Exsiccata examined:

No. *112c. Dasyscypha fuscosanguinea* Rehm on *Pinus sylvestris* in Rehm's personal herbarium at Stockholm. This specimen was collected Prov. Vasterbotten, Jörnsmarken, July 1911 and originally determined by Lagergerg. Spores, elongate elliptic, extreme range (20) $13.0–18.8 \times 4.8–6.8\ \mu$; asci, (10) $104.0–123.4 \times 9.6–11.8\ \mu$. A duplicate specimen in the herbarium, the N. Y. Bot. Garden, gave the following measurements: spores (20) $14.8–22.0 \times 5.0–6.6\ \mu$. Our observations which confirm those of Dear-

ness (2) indicated that these specimens showed agreement with American material of the parasite.

Swedish specimens of diseased Scotch pine (F. P. *53980*—specimen from Lagerberg's Herb.) collected by Lagerberg in Gällivare, Lapland, together with fresh material (*53835*) from the same host, collected by Prof. Hesselman in Tärendö, July 7, 1932 and sent to us for culture study, showed close morphological and cultural agreement with the North American forms collected on three species of five-needled pines.

A Norwegian specimen of the fungus collected by Jørstad (F. P. *64021,* specimen from Jørstad's Herb.) associated with *Phacidium infestans* Karst. on a young dead pine from Fyresdal situated in Telemark fylke. A few apothecia of the fungus were observed present on the needles as well as on the stem where they occurred more abundantly.

A specimen collected by Stillinger, Elk River, Idaho on *Pinus monticola* Aug. 10, 1924, determined as *Dasyscypha monticola* by W. W. Diehl in the Myc. Herb., B. P. I., U. S. D. A., is identical with American material of the parasite listed above.

CULTURE CHARACTERS

As in the *Dasyscyphae* (4, 5) previously reported, ascospores of *D. Pini* germinated readily outside and inside the ascus within 24 hours when plated on 3 per cent malt agar. The spores germinated without septation or commonly became two-celled and produced one, two or more flexuous polar germ tubes. The ascospores were also observed to form two or three septa. After the fourth day, the young, slow-growing hyphae produced by the germinated spores, measured approximately 350–870 μ from tip to tip. A clumped type of colony of very sparsely branched, flexuous hyphae was produced resembling somewhat that produced by *D. Willkommii* spores, except the germ tubes were not pronouncedly "curly" (4, PL. 10, FIG. 5) as in the larch parasite.

A strain of the species growing on *Pinus albicaulis* (*40522*) collected August 31, 1930 was isolated April 29, 1931, after being stored in the ice box for eight months. A strain collected on *P. monticola* (*40731*), August 9, 1931, was isolated after being kept in a like manner for four and six months respectively. On steril-

ized hard oatmeal in Erlenmeyer flasks a dense, whitish, wooly aërial colony, 1 cm. diameter, was formed within a month at room temperature, whereas growth upon the malt agar was even slower. Mono-ascospore strains of collection *40522* produced at room temperature upon 3 per cent malt agar in test tubes within the same period of time, colonies 2–4 mm. in diameter, with well-developed aërial mycelium 6 mm. high, dense, whitish, having the effect of fine combed wool, which with age became tinged with light buff. It was found that while strains of the fungus from North America and Scandinavia would grow at room temperature, test tube cultures kept in the ice box (38°–42° F.) upon sterilized twigs of *Pinus Strobus* standing in hard oatmeal agar, thrived upon this medium. Upon plain oatmeal agar a veritable plug of fine, chalky-white, aërial hyphae 20 mm. high was produced within 3 months, which filled the tube above the medium. Dried out synthetic malt cultures of Scotch pine strain *53835,* which had been stored in the ice box for 18 months, grew vigorously when subcultured upon fresh synthetic malt.

A comparison of culture characters of three strains isolated from *Pinus Strobus, P. monticola* and *P. albicaulis* and the Scandinavian strain from *P. sylvestris* showed satisfactory agreement upon sterilized rice and potato dextrose agar kept at room temperature. Upon the former all the strains formed first a fine whitish, aërial growth, later tinged with buff-yellow. The white rice grains immediately below this aërial growth became a pinkish-buff. On the potato dextrose slants, all the strains grew very slowly. The white aërial growth which formed has a dense, even, circular, pompon-like colony consisting of fine white hyphae which became tinged with buff-yellow about the center of the colony within 15 days in all the tubes except those containing the Swedish strain. However, when the buff-yellow color did appear in the latter it continued to deepen to a beautiful orange-buff, whereas in the American strains the yellow color faded to a pale pinkish-buff. This difference in color reaction between American and European strains appeared to be one of degree among colonies all of which produced essentially the same type of growth.

An imperfect stage of *D. Pini* was never observed among the mono-ascus or -ascospore culture strains growing on the various

media tested. Likewise Lagerberg (**11**) commented upon the fact, that he had failed to observe this stage in the life-history of the fungus.

DISCUSSION

Although *Dasyscypha Pini* was first reported in 1892, the species was not recognnized or collected in this country until 1922. With the discovery of the disease in this country, the striking pathogenic nature of the fungus attracted pathologists, particularly because this canker disease in certain regions was confused with that caused by white pine blister rust (**6**, **15**). Mycologists instinctively sensed that the pathogen involved was very probably distinct, at least physiologically, from Rehm's *D. fuscosanguinea*. Boyce, greatly interested in the fungus, sent material to Washington for identification, which was referred to Dr. W. W. Diehl of the Division of Mycology who examined it critically. Diehl's opinion of the fungus, given in a written communication to the Division of Forest Pathology (Sept. 24, 1923), was as follows: "I have examined with much interest the *Dasyscypha* on *Pinus monticola*. . . . I am unable to find any described species with which this is identical; although the fact is the taxonomy of this genus is not definitely outlined. Your specimen comes nearest to *D. fuscosanguinea* Rehm, but differs plainly from that species in that the paraphyses are definitely branched, not thickened at the apex; the spores are frequently biseptate (as in the genus *Lachnella*); while the sizes of the asci, and even of the spores, are larger. This fungus, in connection with the cankered condition, suggests that of *Dasyscypha Willkommii* Hartig, causing larch canker, although it is definitely a different species. In external appearance this resembles *D. arida* Phill., which occurs on coniferous hosts in the western United States, but this latter species has much smaller spores and asci, and paraphyses with swollen tips."

Two years later Dearness in correspondence (March 25, 1925) with Boyce who had kept the fungus under observation since 1923, expressed the belief that Lagerberg's fungus which he (Lagerberg) had called *D. fuscosanguinea,* was closer to the American brown-excipled parasite, than to the species described and figured by Rehm. To quote Dearness: "If Lagerberg's specimen is correctly named (and I am assuming in the meantime

that it is) your No. *1452* (Herb. J. S. B.) is a vigorous example of that species. Rehm does not imply the species is parasitic. He says it inhabits withered twigs (" durren ästchen ") of *Pinus pumilio* and *P. Cembra*. . . . In my next paper I should like to publish a note of your observations giving it the " brown-red " name on the strength of Lagerberg's specimen and point out discrepancies as by Rehm's description and figures " (2).

To those mycologists averse to the erection of new species, particularly in these days when that practice has become rampant among some workers, the allocation of the fungus described by Brunchorst and Lagerberg as a variety of the Tyrolese saprophyte, a procedure which Stillinger (15) followed, was sufficient to indicate the separate identity of the pine parasite. The authors, however, have preferred to use Brunchorst's name on the basis of the several good morphological as well as physiological characters which tend to set apart the pathogen as quite a distinct fungus.

The question as to whether or not the brown-excipled species belong to the genus *Dasyscypha* also came up for consideration. Nannfeldt (Nannfeldt, J. A., Studien über die Morphologie und Systematik der Nicht-Lichenisierten inoperculaten Discomyceten. Nova Acta Soc. Sci. Upsal. IV. 8^2: 299, 1932) was not disposed to place *D. fuscosanguinea* in the genus *Dasyscypha* (syn. *Trichoscyphella* Nannf.) and stated that within his opinion the systematic position of this species was not clear. When Lagerberg 12, p. 146, FIG. 8) illustrated the anatomical structure of an apothecium of *D. Pini,* he delineated two distinct excipular layers, an outer layer of pseudoparenchymatous tissue consisting of thick walled, brownish cells (" textura oblita ") from which the hairs arose, and an inner layer of loosely-woven, thin-walled, colorless hyphae (" textura intricata "). Inasmuch as the arrangement of the hyphae into layers making up the apothecia of the brown and white-haired species appears to be similar, the authors have retained the brown-excipled forms within the genus *Dasyscypha,* where they have been placed.

It does not appear likely that *D. Pini* was introduced into this country for it apparently has been here many years. The writers have had access to the field notes incorporated in the herbarium of Dr. Boyce, which contain a great deal of valuable information on

the pine canker and its parasite. To quote from Boyce's notes made August 26, 1923: "Found a number of cankers on butt (within 1′–3′ of ground) in an overmature stand, trees 18–30″ D.B.H. and at least 200 years old (Upper Priest Lake, Boundary County, Idaho, Elev. 3600′—parasitic on trees of all ages; most serious on saplings) showing that tree can persist in spite of attack of main stem near ground. These infections resulting in such cankers occurred when the tree was very young with needles on the stem (Resembles photographs of European larch canker; see W. E. Hiley: The fungal diseases of the common larch. Oxford. 1919)." In the case of *D. Pini* we have apparently a fungus belonging to the Arctic flora in Europe, which is also present in this country. The species is to be found in comparatively restricted areas where its peculiar life-habits definitely associated with severe climatologic conditions were possible.

In North America, *D. Pini* has been found in latitudes below those of its habitat in Scandinavia. The Upper Peninsula, Michigan collections, demonstrate that the fungus is able to grow at lower elevations and at a lesser degree of north latitude (see *D. Pini,* Hab. p. 488). In the Pacific Northwest, on the other hand, the fungus is found only at higher elevations. Stillinger (**15**), who gave the more important locations where it has been observed in the western white pine region, wrote: "As a rule, the most heavily infected areas occur at relatively high elevations, that is, 4500′ to 6000′, on sites where white pine is near its altitudinal limits or otherwise on a site rather unfavorable for white pine growth. However, it has been observed in young reproduction in some localities on fairly favorable white pine sites, attacking about 20 per cent of the trees. At lower elevations in other parts of the region, it occurs only as an occasional infection on the smaller limbs, killing these, but causing no other evident damage." The inference to be drawn from these findings is that latitude and elevation play very important rôles with regard to the distribution of the fungus. Moreover, its ability to endure severe climatic conditions, in districts where it readily parasitizes pine, predisposed to disease, particularly in regions representing the distributional borderline of the host species, also must be taken into account in explaining the natural range of the parasite. In the Scandi-

navian pine barrens (11) where the disease occurs, altitude does not play the part it does in northwestern America. Here, as in northern Michigan, it would seem to be a matter of parasitic adaptibility of the fungus in regions where the pine must needs inure itself to very strenuous growing conditions.

Dasyscypha Pini has not been collected in Maine or in other northern New England States in the natural range of eastern white pine. Dr. J. H. Faull, who has collected fungi extensively in Canada, particularly in the Gaspe Peninsula region, did not come upon it. Dr. Baxter, who made pathological observations and collected this parasite at Gällivare, Lapland in 1930 (Baxter, D. V., Observations on Forest Pathology as a part of forestry in Europe, Univ. Mich. Forest & Conserv. Bull. No. 2: 32, 1933) did not find the fungus parasitizing *Pinus contorta* in Alaska in the regions visited during two collecting trips in 1932 and 1933.

In consulting maps that give the mean temperature in degrees Fahrenheit, for the month of January in different parts of the world (A. J. Henry and others, Weather and Agriculture, U. S. D.A. Yearbook 1924, pp. 457–558), it will be noted that practically the same isotherms, 25°–30°, pass through the Pacific Northwestern, Upper Peninsula, Michigan and Scandinavian localities where *Dasyscypha Pini* has been recorded. And again if one consults the mean July temperature chart one finds a somewhat similar condition of climatic agreement indicated by isotherms 50°–65°, although the summer agreement in temperature is not so striking.

A consideration of the isolated distribution of *Dasyscypha Pini* offers interesting ground for speculation. Fernald (3) has pointed out that segregations of higher plants in the northern hemisphere is shown by groups, often identical species, which occur in Europe and Pacific North America but are absent from eastern Asia and eastern America. Obviously climate is largely concerned in accounting for the segregation of plant species. In the case of *D. Pini,* low temperatures are undoubtedly correlated with the ability of the fungus to parasitize pine growing with difficulty upon unfavorable sites. Average precipitation is probably another factor for consideration. These factors, together with elevation and latitude, must be taken into account in explaining the occurrence of this peculiar parasite limited to the host genus *Pinus*.

The reader raises the question, "Why has not *Dasyscypha Pini* been found among the white pine stands of New England, if not as a parasite, at least as a saprophyte?" It may be as Fernald pointed out in the case of the higher plants, that *D. Pini,* an Arctic species (granting that it is to be considered as such), may have become isolated and is so ancient as to have lost the capacity of pioneering. We know nothing of the antiquity of *D. Pini* with respect to past geologic ages. On the other hand, it may be that instead of dealing with an old species, we may be considering one comparatively newly evolved. These new forms Bisby (Bisby, G. R., The distribution of fungi as compared with that of phanerogams, in Am. Jour. Bot. 20: 246–253. 1933) suggests, have not yet had time to spread to or become adapted in the more distant host ranges. As that writer has stated, the distribution of parasites, generally speaking, is more limited than that of saprophytes, and their spread depends primarily on the distribution of their hosts. The introduced parasites, white pine blister rust (*Cronartium ribicola* Fischer) and chestnut blight [*Endothia parasitica* (Murr.) P. J. & H. W. And.] are examples of wide distribution with relation to that of their respective hosts. In the case of *D. Pini,* however, we may be dealing with a parasite peculiarly specialized as regards the necessary environment for its existence, and for this reason limited in its present distribution.

So far as the authors know, *Dasyscypha fuscosanguinea* is restricted to the genus *Pinus* of central Europe. It has not been reported from Scandinavia, particularly in those localities where *D. Pini* has been found. The former species, according to Rehm is a high elevation species and would appear to be distinct from the shorter-spored saprophytes, *D. flavovirens* on larch from the Tirol and the widely distributed western American species, *D. arida* occurring on a number of conifers. The determination of the relationship of these saprophytes and the decision as to whether or not *D. fuscosanguinea* and *D. flavovirens* occur in North America, remain for further investigation.

Our investigations (3) with *Dasyscypha Willkommii* showed that the European larch parasite was to be found fruiting in immediate association with the lesions which it produced and that the saprophyte, *D. calycina* Fuckel, then came in as a secondary

organism to colonize the weakened and dying parts killed by the parasite. In a like manner, *D. Pini* is only to be found in the immediate association with the cankers which it produces. More or less limited to these cankers, the fungus continues to fruit abundantly, even after the branch or part upon which the lesions occur is dead. The fungus in America has not been observed to spread out along the branch as a saprophyte in the manner of *D. calycina.* Boyce, who made a study of the parasite in the Pacific Northwest, orally confirmed the authors in this observation. It is of interest here to note that in Sweden, Lagerberg (11, pp. 152–3) reported *Lachnellula chrysophthalma* (Pers.) Karst. as frequently occurring with *D. Pini* on living pine. However, he noted that *L. chrysophthalma* was to be found only as a saprophyte on the upper part of the stem which had been girdled and killed by the attack of the parasite below. The authors have confirmed this association of parasitic and saprophytic species, in northern Michigan specimens of diseased *Pinus Strobus.*

SUMMARY

Investigation of a large-spored, brown-excipled *Dasyscypha* species, associated with a destructive canker of native *Pinus Strobus, P. monticola* and *P. albicaulis* in North America and on *P. sylvestris* in northern Scandinavia, has shown the pathogen to be distinct from the innocuous saprophyte, *D. fuscosanguinea* Rehm (1881), with which it hitherto has been regarded as synonymous.

The highland parasite is referred to Brunchorst's species *Lachnella Pini* (1892) with the new combination, *Dasyscypha Pini* (Brunchorst) Hahn & Ayers. Morphological and physiological data are presented to support the separation of Brunchorst's parasite from Rehm's Alpine saprophyte of central Europe. Culture characters of *D. Pini* are given.

D. Pini appears to have a circumpolar distribution, and is to be found only at north latitudes and usually at high elevations (3000′–6000′) on cold mountain slopes or in Arctic pine barrens where climatologic conditions are exceedingly severe. A phytogeographical discussion is given.

Since Brunchorst gave the first description of the disease, very

little further study has been made of it in Norway. Lagerberg in Sweden contributed valuable pathological and mycological data with respect to the fungus. In North American the fungus was reported for the first time in 1926. Although it is now known to have been present in the United States for many years, generally distributed over the western white pine region of the Inland Empire at high elevations, the first collection of the fungus was not made until 1922. The disease has been confused with white pine blister rust in the Pacific Northwest; in the Upper Peninsula, Michigan, where it has recently been found, it presents a similar problem. A culture study has shown that North American forms isolated from the three native hosts show satisfactory agreement with isolations from Swedish material collected on Scotch pine at Tärendö in the Arctic region.

The canker caused by *D. Pini* is very similar to that caused by *D. Willkommii* (Hartig) Rehm on larch species. The fruiting cups of the former are always to be found, as in the case of the latter, in immediate association with resinous lesions in which the pathogen continues to fruit even after the branch is killed. The organism does not spread saprophytically out into the killed branch beyond. In Scandinavia, *Lachnellula chrysophthalma* (Pers.) Karst. may colonize such dead parts, in this respect resembling the saprophyte *D. calycina* Fuckel, which colonizes branches killed or weakened by the European larch canker parasite.

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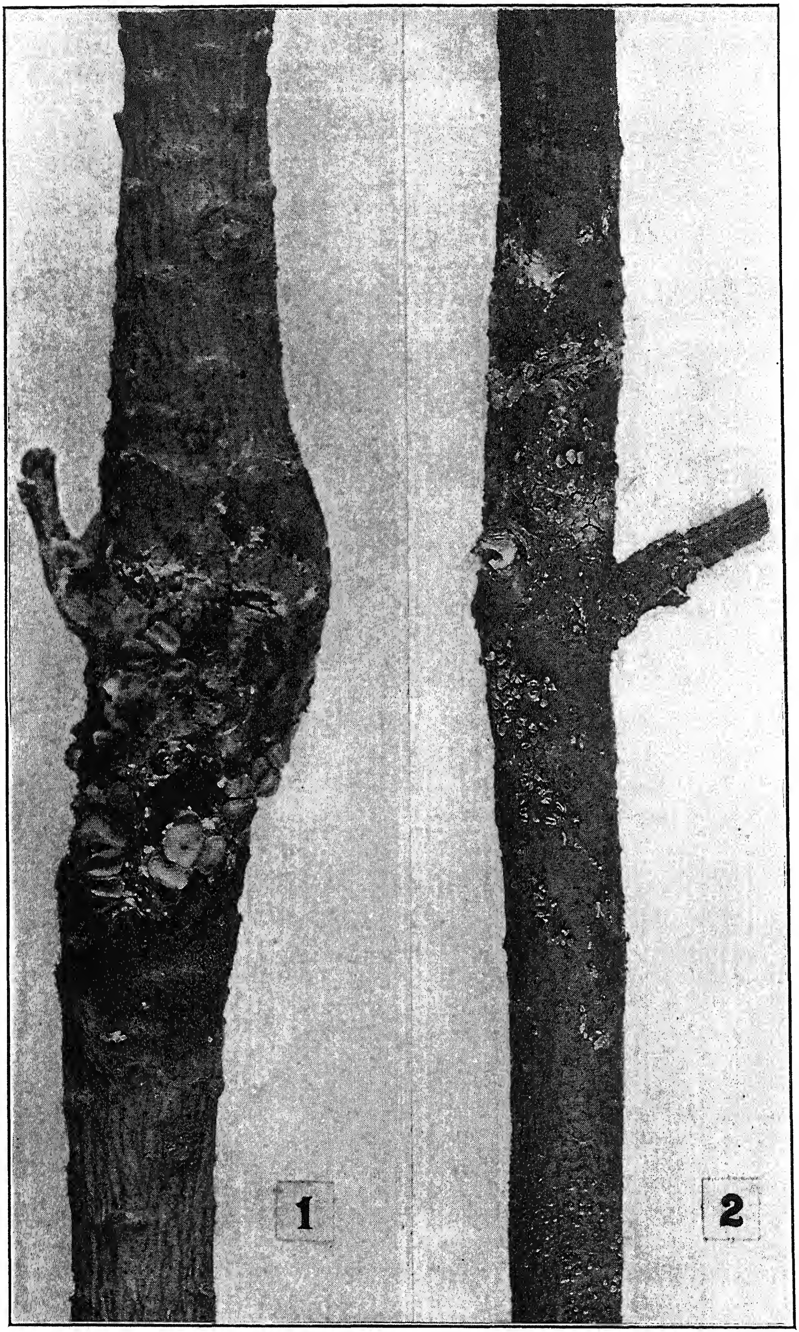

Dasyscypha Pini

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EXPLANATION OF PLATES

PLATE 52. *Dasyscypha Pini* (Brunch.) Hahn & Ayers

Canker on living branch of *Pinus albicaulis* showing the brown-excipled apothecia with bright orange hymenia of *D. Pini* (*40521*). This specimen was collected Mt. Revelstoke Nat'l Park, British Columbia, elev. 4000′–5000′, 50° 45′ n. lat., August 24, 1930. (Approx. 4 × nat. size.)

PLATE 53. *Dasyscypha Pini*

Fig. 1, Canker on living branch of *Pinus albicaulis* (*40521*) associated with the fruiting bodies of *D. Pini.* The parasite continues to fruit in the canker after the branch has been girdled and parts above killed. (Approx. 2 × nat. size.)

Fig. 2, Canker on heavily infected *P. Strobus* (*53083*); the branch has been killed both above and below the lesion by *D. Pini.* Apothecia are shown upon the lesion fruiting in the canker. Note fruiting bodies of a secondary organism, *Coccophacidium crustaceum* (Curt.) Durand which has colonized the dead cortex below the canker. (Slightly below nat. size.)

MYCOLOGICAL NOTES FOR 1933 [1]

L. O. Overholts

(with plates 54 and 55 and 1 text figure)

FUNGI IMPERFECTI

1. Ascochyta lethalis Ellis & Barth.

What appears to be the first Pennsylvania collection of this species on *Melilotus officinalis* was made near St. Marys, Elk County, Pennsylvania. The infestation was very heavy on the stems and great damage to the crop was apparent.

2. Cercospora Menispermi Ellis & Holw.

Collected on leaves of *Menispermun canadense* in Huntingdon County, Pennsylvania, in 1930.

The original description calls attention to the preponderance of short spores in this species, 15–25 × 5–7 μ. Tehon (Mycologia **16**: 138. 1924) describes some spores as much elongated (to 140 μ) for specimens from Illinois. The spores of my collection agree better with the shorter spores of the original description, measuring 18–52 × 4–6 μ.

3. Cornularia macrospora (Berk. & Curt.) Sacc.

Collected in Center County, Pennsylvania, in 1928, on dead branches of *Robinia pseudacacia*. Pycnidia columnar, black, about 1 mm. high, 120–180 μ diameter; spores elongate, hyaline, attenuate at both ends, several-celled, 55–65 × 2.5–3 μ (plate 55, fig. 9, 13).

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, March 12, 1934, as Technical Paper No. 640. Contribution No. 97, Department of Botany, The Pennsylvania State College, State College, Penna.

For the last previous article in continuation of this series see Mycologia **25**: 418–430. 1933. I am indebted to Dr. J. Dearness, Miss Edith Cash, and Dr. W. W. Diehl for some of the determinations herein reported.

4. DIMEROSPORIUM BALSAMICOLA Peck.

This much named fungus was collected on dead leaves of *Abies balsamea* at Highlands, North Carolina, in August, 1933. It fruits abundantly on the lower leaf surfaces and sparingly on the upper surfaces. It seems to be not often collected and this probably extends its range far southward from previously reported stations. The fungus is past the sporulation stage but the pycnidia are very characteristic, especially in section.

5. DIPLOCLADIUM PENICILLIOIDES Sacc.

A species of *Diplocladium,* referred provisionally to the above species was collected in Armstrong County, Pennsylvania, in 1932. The fungous host was so much disfigured that even a generic reference was impossible in the dried condition, though the indications pointed to a species of *Pleurotus.*

Forming a soft compact mat over the gills of the substratum, white or in older portions verging toward salmon or pale orange; conidiophore hyphae extensively branched, the ultimate branches more or less verticillate, 2–4 in number, 50–70 × 4–6 μ, tapering to a point and bearing a single terminal spore; spores ovoid to ellipsoid or oblong-ellipsoid, smooth, hyaline, 2-celled, 12–18 × 6–9 μ (PLATE 55, FIG. 7).

6. HETEROSPORIUM ALLII Ellis & Mart.

Causing extensive injury to leaves of wild garlic, *Allium canadense,* in Lancaster County, Pennsylvania, in June, 1933. Not previously collected in the state, but originally described from New Jersey. Spores minutely verrucose, 24–36 × 10–12 μ, mostly 2–4-celled.

The variety *Allii-Porrii* Sacc. & Berlese was collected on *Allium* at Mt. Union, Huntingdon County, Pennsylvania, in 1929. In it the spores are 42–66 × 12–15 μ.

7. LEPTOSTROMELLA FILICINA (Berk. & Curt.) Sacc.

Seldom reported, but probably common on dead overwintered leaf stalks of various species and genera of Filicales. Material in excellent condition was collected in Center County, Pennsylvania, in 1926 and again in 1932.

Pycnidia crowded, often confluent, somewhat elongate, shiny-

black, sometimes as much as 0.5 mm. long, usually smaller, about 200 μ broad, sub-epidermal in origin and blackening the epidermal cells; conidia linear, curved or crescent-shaped, hyaline, 3–5-celled, 20–40 $\times$ 1–1.5 μ.

On dead overwintered leaf stalks of *Osmunda* and *Onoclea*.

8. LEPTOTHYRIUM PERICLYMENI (Desm.) Sacc.

The first Pennsylvania collection was made June 13, 1933, on *Lonicera canadensis*. The following notes were made:

Spots definite, circular, most conspicuous from the upper surface, 3–6 mm. diameter, the centers becoming ashen, the margin remaining red-brown; pycnidia epiphyllous, lenticular, scattered, 4–15 per spot, 120–160 $\times$ 40–60 μ, in section showing a definite black roof and a hyaline hymenial layer, apparently subcuticular in origin; conidiophores short, inconspicuous; conidia retort-shaped, *i.e.* enlarged at the basal end and tapering to an attenuated and laterally bent tip which seems to be cut off from the main body of the spore by a septum, otherwise 1-celled, hyaline, 10–13 $\times$ 7.5–8 μ exclusive of the tip, 16–18 $\times$ 7.5–8 μ measured along the chord to the tip. (Compare *Kabatia Periclymeni* (Desm.) Bubak.)

One might expect the attenuate spore tip to be the spore-pedicel but such is not the case (PLATE 55, FIG. 12).

9. PHYLLOSTICTA MACROSPORA Ellis & Ev.

Collected on living leaves of *Liriodendron Tulipiferae* in Armstrong County, Pennsylvania, in 1932. It inhabits spots that on other occasions harbor *P. Liriodendri* and occasionally some of Moniliales such as *Alternaria* and *Cercospora*.

Spores elongate, subfusoid to almost oblong with ends slightly pointed, 1-celled, smooth, hyaline, 16–22 $\times$ 4–6 μ. It does not seem probable that this species and *P. Liriodendri* could be synonymous.

10. RAMULARIA SAMBUCINA Sacc.

On living leaves of *Sambucus racemosa*, Sullivan County, Pennsylvania. Spots 2–3 mm. diameter, black above, paler and concave below. Conidiophores hyaline, up to 45 μ long, 2.5–3.5 μ diameter, in clusters of 15 or more; conidia cylindric, the ends slightly narrowed, 15–30 $\times$ 2.5–3.5 μ.

R. sambucina Peck is probably the same, though described as having spores 5–6 μ diameter.

11. RAMULARIA SHELDONI Trotter. (= *R. Delphinii* Dearness & House).

Collected at the U. S. Gold Corporation above Eldora, Colorado, elevation 10,000 feet, July 31, 1926, on *Delphinium* sp. Spores 15–24 × 5.5–6 μ. Originally described from Gray, Colorado.

12. SEPTORIA INCRESCENS Peck.

On leaves of *Trientalis americana*, Walden, Vermont, 1917. Spores 16–36 × 1–1.5 μ, straight, 2–4-celled. Pycnidia epiphyllous, minute.

13. SEPTORIA MIMULI Ellis & Kellerm.

On living leaves of *Mimulus ringens*. Huntingdon County, Pennsylvania, 1932. Spots sub-circular, 3–6 mm. broad, of which the major portion is the broad reddish-purple margin; pycnidia amphigenous, 60–80 μ diameter; spores linear, several-celled, 25–40 × 1.5–2 μ.

14. STEGANOSPORIUM ACERINUM Peck.

S. piriforme Hoffm. has been several times collected in Pennsylvania in recent years, but *S. acerinum* but once, and it probably is not common. It differs from the first named species in the larger spores, 45–60 × 27–31 μ, and probably in the habit of extruding them in comparatively immense tongue-like bands as much as 5 mm. broad. Potter County, Pennsylvania. On dead *Acer Saccharum*. September, 1925.

15. TOXOSPORIUM ABIETINUM Vuill.

Collected on dead leaves of *Abies balsamea* in company with *Dimerosporium balsamicola* at Highlands, North Carolina, in August, 1933. The acervuli, so far as observed in sections of this material, are merely minute clusters of spores, apparently subcuticular in origin and not discernible except by most careful study of leaf sections. Spores, however, are obtainable in crushed mounts from scrapings of the leaf surface. Peck seems to have described this as *Pestalozzia* (?) *campsosperma*, which was transferred to *Monochaetia* in the Sylloge. I have not studied the type material but the indications are that Peck obtained spores in micro-

scopic mounts and erroneously assumed them to be produced in the pycnidia of an accompanying *Dimerosporium balsamicola.*

ASCOMYCETES

16. GODRONIOPSIS QUERNEA (Schw.) Diehl & Cash.

Two collections by C. S. Moses, near Voluntown, Connecticut, extend the range of this species slightly. They were taken from living branches of *Quercus coccinea.* The excipular ridges mentioned by Diehl and Cash as a feature of the species are not prominent and would be mistaken for corrugations due to drying if specimens were not soaked up. The ascospores measure only 25–32 × 6–8 μ in my preparations, but Miss Cash agrees that the fungus is correctly referred here. The outer layer of the exciple, showing black in sections, becomes green in KOH solution and a drop of KOH containing an apothecium becomes a dirty red-brown color.

17. MYCOSPHAERELLA IMPATIENTIS (Peck & Clinton) House.

Collected in Cook Forest, Clarion County, Pennsylvania, in 1927, on *Impatiens.* Forming indefinite, inconspicuous brownish spots 4–10 mm. diameter, not sharply delimited; perithecia amphigenous, numerous, thickly gregarious, 50–80 μ diameter; asci saccate to clavate, 8-spored, 45–58 × 8–12 μ; spores elongate, smooth, hyaline, 2-celled, 10–14 × 3–4 μ

18. MYCOSPHAERELLA THALICTRI (Ellis & Ev.) Lindau.

Collected on living leaves of *Thalictrum,* in Armstrong County, Pennsylvania, in 1932. Forming small white spots 2–3 mm. long, with a very narrow darker border; perithecia epiphyllous, numerous, 60–80 μ diameter; spores biseriate, elongate, 2-celled, 9–13 × 3–3.5 μ.

19. OCELLARIA AUREA Tul.

Answering to this description is a fungus collected on dead branches of *Salix* in Armstrong County, in 1932. Apothecia 300–500 μ diameter, erumpent, opening widely, with a definite rim-like margain, it and the hymenium golden brown in color; asci 80–115 × 20–25 μ, 8-spored; spores ellipsoid, smooth, hyaline or somewhat golden, 22–27 × 11–13 μ (PLATE 55, FIG. 14, 15).

20. PSEUDOPEZIZA AUTUMNALIS (Fuckel) Sacc.

Two Pennsylvania collections have been made of this species, one in Forest County in 1921, and one in Center County in 1933, the latter on *Galium circaezans.* Apothecia 60–100 μ diameter, grouped on yellowish spots, hypophyllous; spores narrow-fusoid or cylindric with pointed ends, $7–9 \times 2–2.5\ \mu$.

BASIDIOMYCETES

USTILAGINALES

21. ENTYLOMA ARNICALE Ellis & Ev.

Collected at an elevation of 10,000 feet, above Eldora, Colorado, July 31, 1926, on *Arnica cordifolia.* The measurements of both chlamydospores and conidia under-run those given by Clinton, the former being 9–14 μ diameter and the latter 10–20 μ long. Clinton lists the species only from Idaho and Washington. *Ramularia arnicalis* Ellis & Ev. is the conidial stage of this smut and was collected at Rimini, Montana, which adds still another state to the known range.

UREDINALES

22. PUCCINIA PARCA Arth.

A rust of limited distribution on the Atlantic coast and apparently not frequently collected. A collection was made at the Gate-keeper's lodge at the foot of Mt. Mitchell, Black Mountain, North Carolina, in August, 1933. The species seems easily recognizable in the telial stage by the hyaline papillae projecting over the germ pores. Both uredinia and telia are present on this material.

23. PUCCINIA TENUIS (Schw.) Burrill.

Orton in the North American Flora records this species as occurring southward only to West Virginia. A collection made by the writer at the foot of Mt. Mitchell, North Carolina, in August, 1933, is therefore an extension of its range in that direction. Both aecia and telia are present on leaves of *Eupatorium urticaefolium* collected at the Gate-keeper's lodge at the entrance to the Mt. Mitchell road at Black Mountain. The teliospores are as much as 40 μ long, exclusive of pedicel.

24. PUCCINIASTRUM AMERICANUM (Farl.) Arth.

Reported in the North American Flora as ranging south to West Virginia. I collected it on *Rubus idaeus aculeatissimus* (= *R. strigosus*) atop Mt. Mitchell, North Carolina, in August 1933.

25. PUCCINIASTRUM HYDRANGEAE (Berk. & Curt.) Arth.

Collected on *Hydrangea radiata* at the upper falls of the White Water River, Transylvania County, North Carolina, in August 1933. This seems to be an unreported host for the species which has been known only on *H. arborescens* in the uredinial and telial stages.

HYMENOMYCETALES

26. ALEURODISCUS AMORPHUS (Pers.) Rabenh.

Collected at Cheat Mountain, West Virginia, on dead *Abies balsamea*, June 8, 1933. This extends the range of this species southward from New York to West Virginia. The spores, as I usually find them, are nearly or quite smooth.

27. ALEURODISCUS APICULATUS Burt.

A collection answering fairly well to Burt's description of this species was taken at Highlands, North Carolina, in August, 1933. The only departures from Burt's description are in the following points: in structure 400–600 μ thick instead of 600–800 μ; paraphyses (with lateral prongs) measuring 5–12 μ diameter rather than 6–7 μ; basidia up to 20 μ diameter instead of 12–15 μ; sterigmata 15–20 μ long rather than 15 μ; spores 24–28 $\times$ 12–16 μ, rather than 20–25 $\times$ 12–15 μ. While Burt's type was collected in Jamaica, yet it was at an elevation of 5,000 ft., so that the discrepancy in geographical range is not so great. I have been unable to see definite echinulations on the spores of my collection, but those species of *Aleurodiscus* that have roughened walls seem to develop such roughness relatively late and Burt remarks that two other collections seem by him from Grenada and from Porto Rico had even-walled spores and were probably immature. The shape of the spore is so highly characteristic that if my collection does not represent his species it must be quite closely related to it. The color of the hymenial surface is about that of *Corticium roseum*. Reported by Coker from North Carolina in 1927.

28. BOLETUS BETULAE Schw. and B. RUSSELLII Frost.

In 1902 Beardslee maintained (in Lloyd, Myc. Notes p. 97, 1902) that these two were phases of the same species, the differences previously noted being due to weather conditions. Murrill, however (American Boletes p. 8, 1914) maintained the two species, describing one as tomentose and with longitudinally striate spores and the other with viscid glabrous pileus and papillate spores. I collected both species in the same woodlot near Marion, North Carolina this summer. There can be no doubt but that the two species are distinct, the differences following along the lines laid down in Murrill's description. The spores in particular present a fine point of separation in dried plants.

29–32. CORTICIUM—Section Botryodea.

This section of *Corticium* seems to be a natural group of species characterized by the hypochnoid and tender structure, pale color, a more or less discontinuous hymenium that appears granular under a lens, the granules representing clusters of basidia borne in a somewhat botryoid fashion on the apexes of short lateral branches of the coarse and septate subiculum hyphae. The basidia are much broader in proportion to their length than in the usual clavate type of basidium. They differ also in often producing up to eight sterigmata on each basidium. The subiculum hyphae are very loosely arranged, of broad diameter, and with right-angle branching.

Burt recognized two species in this complex, the common *Corticium vagum* and another described under the name *Coniophora vaga*. In addition to these two I have in my herbarium American specimens of *C. subcoronatum* and specimens of a different species I am proposing as new at this time. These species are to be differentiated as follows:

Subiculum hyphae with clamp connections, 5–8 μ diameter; spores narrow-ovoid or narrow-ellipsoid and flattened on one side, to nearly cylindric and pointed at one end, 5–7 $\times$ 2–3.5 μ; basidia 5–6 μ diameter. *C. subcoronatum.*

Subiculum hyphae without clamps.

- Subiculum hyphae reaching diameters of 12–14 μ or more; spores broadly ovoid or subglobose, 3.5–5 $\times$ 3–4 μ diameter; basidia 8–10 μ diameter .... *C. botryoideum.*
- Subiculum hyphae not more than 8–10 μ diameter. Spores ovoid to ellipsoid, apiculate, 6–8 $\times$ 4–5.5 μ .... *C. fenestratum.*
  - Spores more elongate 7–9(—12) $\times$ 3–4.5 μ .... *C. vagum.*

29. **Corticium botryoideum** sp. nov.

Effused as a thin hypochnoid layer, separable in small bits when wet and then tender in texture, smoky-gray to pale olive buff (Ridgway) or finally deep olive buff; in section 120–300 μ thick, composed of loosely arranged suberect hyphae 6–18 μ diameter, divided into short cells, without clamps at the septa, hyaline or nearly so or a few of those of largest diameter somewhat brownish, branching at nearly right angles, the branches ascending and terminating in short-cylindric basidia 8–10 μ diameter, with 4–8 sterigmata 3–5 μ long at maturity; spores broadly ovoid or subglobose, smooth, hyaline, 3.5–5 $\times$ 3–4 μ; cystidia and gloeocystidia none.

On the bark of prostrate limbs of deciduous trees: noted on *Acer, Alnus, Betula, Carya, Fagus,* and *Hamamelis.* Type collected at Biglerville, Adams County, Pennsylvania, on dead *Alnus rugosa,* July 26, 1932. (Overholts Herb. No. *14503.*) Other collections, all from Pennsylvania, are at hand from Jefferson, Sullivan, Huntingdon, and Armstrong Counties (PLATE 55, FIG. 10).

30. **Corticium fenestratum** nom. nov. (*Coniophora vaga* Burt; not *Corticium vagum* Berk & Curt.).

I have examined the type collection of *Coniophora vaga* and find the spores so dilutely colored that there exists no logical reason for separating the species into a different genus when its relationships are so obviously close to this section of *Corticium.* I measure the hyphae in the type collection somewhat broader then given by Burt. The broadly ovoid and rather strongly apiculate spores, and hyphae of smaller diameters (7–9 μ) make this species distinctive (PLATE 55, FIG. 5).

31. CORTICIUM SUBCORONATUM von Höhn. & Lit.

Forming a thin gray or slightly yellowish-gray discontinuous pellicle 40–80 μ thick on the surface of rotted wood, appearing velutinate under a lens, delicate and separable; in section composed of a few loosely interwoven hyphae along the substratum, these sending up branches of smaller diameter, all hyaline or nearly so, thin-walled, 5–8 μ diameter, with rather abundant clamps of rather small size for such large hyphae; spores narrow-ovoid or narrow-ellipsoid and flattened on one side, to nearly cylindric and pointed at one end, 6–7 $\times$ 2–3.5 μ; basidia 5–6 μ diameter, 4–8-spored.

On rotten wood, usually of coniferous trees. Specimens are at hand from Massachusetts and Pennsylvania.

The clamped hyphae set this species off from its relatives. For comparison, I have the species as communicated to me by Dr. Wakefield of Kew (PLATE 55, FIG. 11).

32. CORTICIUM VAGUM Berk. & Curt.

This species is easily recognized if in sporulating condition by the fusoid or *Euglena*-shaped spores. The hyphae are of small diameter (6–9 μ) and are without clamps at the frequent cross walls. It is a common species on the under side of logs of both coniferous and deciduous trees. At times it apparently revives for two or three years and then attains a thickness of 400 μ and shows a compactness of structure that is at variance with its usual thin hypochnoid condition. About 50 collections are in our herbaria (PLATE 55, FIG. 4).

33. CYPHELLA CARICINA Peck.

A good collection of this species was made in May 1933, growing on dead stems and leaves of *Carex* and *Juncus* in a swampy area near State College. The species is otherwise apparently known only from the type collection in New York. We present a photograph obtained from this material (PLATE 54, FIG. 3).

34. LENTINUS HAEMATOPUS Berk.

Pileus 4–7 cm. broad, tough, reviving well, circular to subreniform in outline, somewhat depressed to somewhat infundifuliform, the margin frequently wavy or lobed, thin, somewhat hygrophanous and pale watery-brown (close to " cream buff " or pale " chamois ") when moist, ochraceous on drying, slightly radiately ribbed at certain stages of drying; context concolorous, tough and somewhat cartilaginous, thin, tasteless, but with a strong anise odor that is only slightly apparent in revived specimens; gills pallid or ochraceous, subdistant, unequal, 2–3 mm. broad, anastomosing on the stem, minutely serrated on edge, the sides covered by resinous-appearing hyphal pegs; stem central or excentric, short but distinct, 0.5–1.5 cm. long, 4–8 mm. thick, dark red, smooth or slightly rugose, glabrous or slightly furfuraceous, solid; spores cylindric, hyaline, 6–8 $\times$ 2.5–3 μ; cystidia none.

This is evidently a rare species. Peck reported two collections from New York and Kauffman found it once in Michigan. Aside from the types collected in North Carolina, these seem to be the only recorded stations. It was collected in Sullivan County, Pennsylvania, in July 1932. It is a distinct and well marked species, but might be sought in the genus *Panus*. It would seem a bit doubtful whether *P. anisatus* Henn. as distributed in the Pennsylvania State College copy of Sydow's Myc. March is the same thing (TEXT FIG. 1).

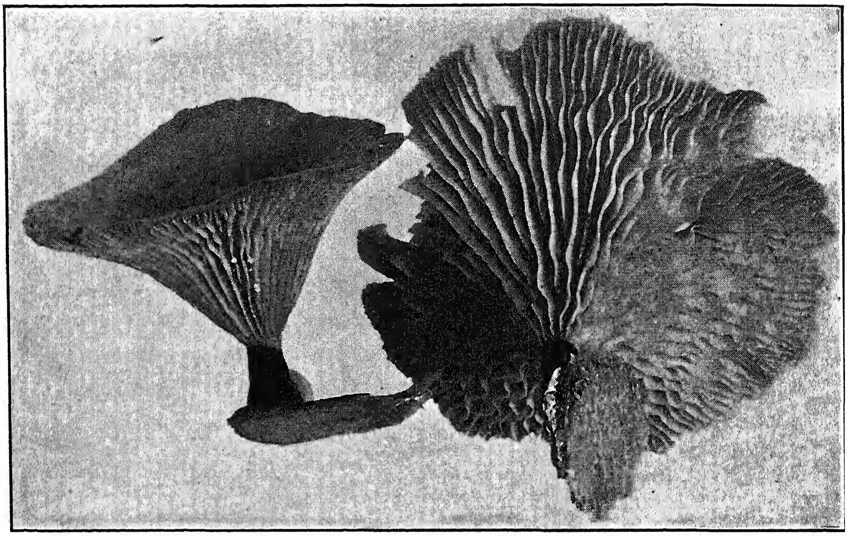


FIG. 1. Photo of *Lentinus haematopus*. × 1.

35. LEPIOTA BRUNNEA Farl. & Burt.

This is apparently the American form of *L. rhacodes*, differing in the browner pileus and somewhat larger spores. Zeller found what he considers the true *L. rhacodes* on the west coast. It has been reported only a few times in the east and probably all these eastern collections are referable to *L. brunnea* if that species is to be maintained. A fine collection was made near State College, September 7, 1933 (PLATE 54, FIG. 1, 2).

36. PISTILLARIA ABIETINA Fuckel.

Erumpent through the outer bark, first as small brown lobulate sclerotia, or the sclerotial base remaining immersed, giving rise

to one or several strongly clavate to pileate basidiocarps less than 1 mm. high, sometimes flattened and spathulate, yellowish or ochraceous, drying hard, fleshy when fresh, not at all waxy or gelatinous; basidia 4-sterigmate; spores cylindric or cylindric-ellipsoid, smooth, hyaline, 7–10 × 3–4.5 μ; cystidia none, hyphae 3–4 μ diameter, with clamps.

On dead twigs of *Pinus Strobus*. Norwich, New York. Collected by R. W. Davidson, in April, 1933.

Unquestionably referable to the genus *Pistillaria* but doubtfully referred to this species. There is at least a general agreement with the picture presented by the available descriptions. The description presented by Bourdot and Galzin seems adequate enough to cover our specimens. They record the spores as 9–11 × 4–6 μ, and the hyphae 3–5 μ diameter.

37. **Peniophora delectans** sp. nov.

Resupinate, effused as a very thin, indistinct, subpruinose, indeterminate, gray-cinereous or gray-caeruleous film; in section 20–25 μ thick, consisting of scarcely more than a basidial layer seated on the substratum; basidia hyaline, 4 μ diameter, 4-spored; spores cylindric, smooth, hyaline, 4–5 × 1.5–2 μ; cystidia abundant and conspicuous in lactic acid mounts and then erect, straight, cylindric, projecting up to 40 μ, with walls entirely thickened except for an enlargement of the lumen at the apex of the cystidium, 5–8 μ diameter, some sparingly incrusted at the tips; in KOH they are indistinct, easily overlooked, collapsed, bent, etc.; where best seen in lactic acid they extend to the substratum.

On dead wood of coniferous trees. Type collected in Cook Forest, Clarion County, Pennsylvania, June 23, 1932. (Overholts Herb. No. *16260*.)

There seems to be nothing like this described. When KOH sections are first examined the partially destroyed cystidia are so inconspicuous that one refers the species to *Corticium*. In lactic acid the cystidia are so numerous and so conspicuous and the subiculum so inconspicuous that one thinks for a moment that the cystidia are foreign to its structure. The small lumen, abruptly enlarged at the apex of the cystidium places this species in the series with *P. crassa* and *P. glebulosa*. *P. subalutacea* seems to approach nearest but that has thin-walled cystidia and the color of

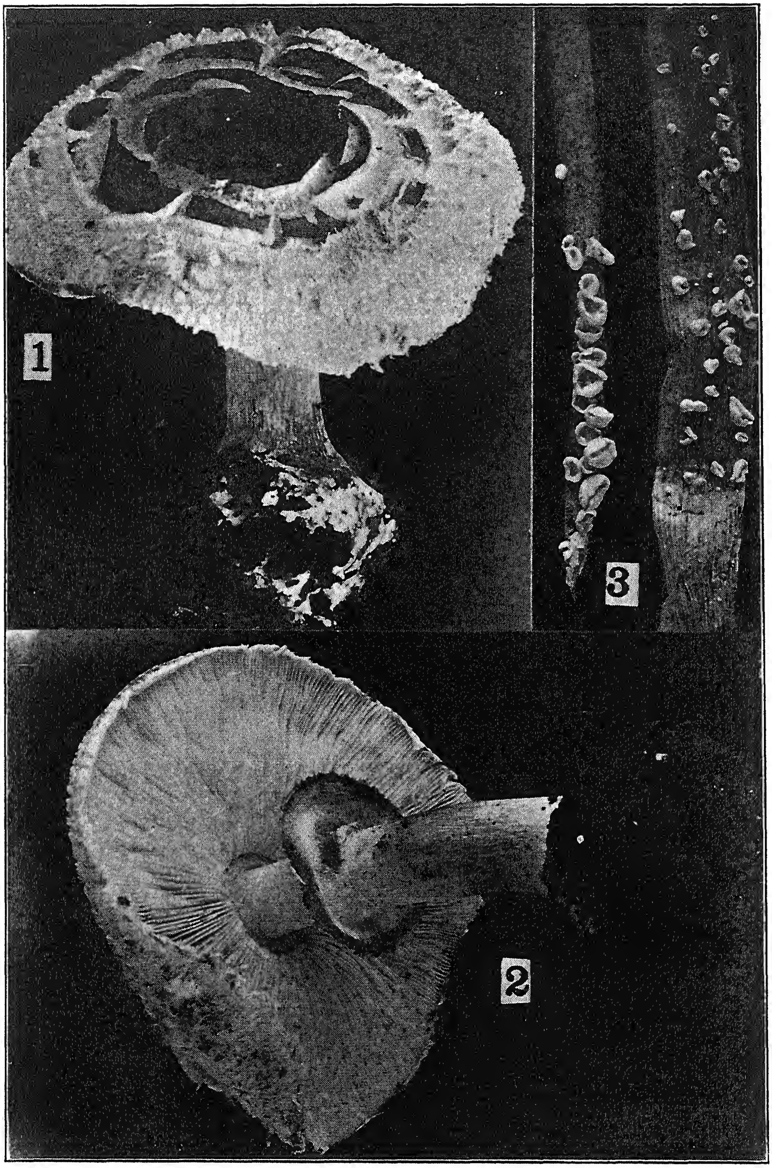
the hymenial surface is very different. *P. pilosa* Burt presents some of the characters of this species but the cystidia there are thin-walled throughout and the spores are larger. *P. dissoluta* Overh. of this paper has much larger and broader cystidia that distinctly taper to a point and are thick-walled throughout. These two species and *P. glebulosa* are the only American species in which the cystidia dissolve and disappear in KOH (PLATE 55, FIG. 8).

38. **Peniophora dissoluta** sp. nov.

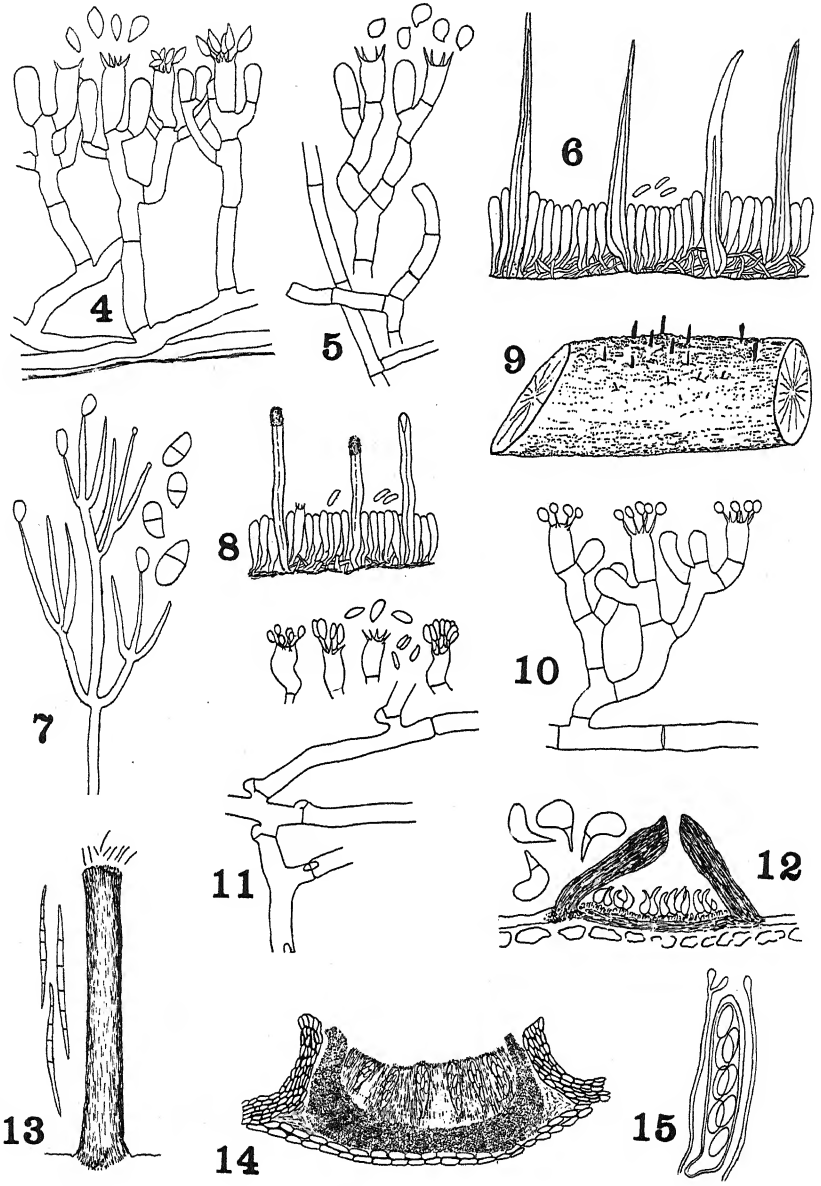
Widely effused as a thin sub-pruinose, discontinuous, pale olive gray to pale vinaceous fawn (Ridgway) film, shortly pilose under a lens, the margin indeterminate; in section 25–40 μ thick, composed almost entirely of the basidial layer, but with a very thin subiculum that becomes more visible beneath the slight elevations in the hymenial surface, and then seen to be of colorless and unincrusted hyphae; spores cylindric, hyaline, $4–5 \times 2\ \mu$ (not seen attached to basidia); cystidia quickly dissolved in KOH solution leaving nothing but the very narrow lumen contents, but undissolved in lactic acid and then seen to be very numerous, elongated, tapering to a rather sharp point, not at all or only slightly incrusted, originating at the substratum, thick-walled with a very narrow lumen that is not enlarged at the apex of the cystidium, $120–160 \times 8–11\ \mu$, projecting for most of their length and often somewhat curved; gloeocystidia none.

On dead decorticated wood of deciduous trees. Type collected at Musser Gap, Center County, Pennsylvania, April 25, 1933. (Overholts Herbarium No. *16264*.)

In Burt's classification this species would seem to belong in the *P. crassa*—*P. glebulosa* group, although the cystidia are not cylindric. *P. pilosa* Burt has thin-walled cystidia of much smaller size, usually terminated by moniliform bodies, not dissolved in KOH, and the spores are somewhat different. *P. Albugo* Burt, in another group, has smaller cystidia that are not dissolved in KOH and the spores are larger. *P. delectans* Overh. of this paper has cylindric cystidia that are incrusted at the apex and the lumen is there enlarged as in the *P. crassa* group. When sections are mounted in KOH, and even before one can examine them under the microscope the walls are dissolved, leaving only the bent and



1, 2, LEPIOTA BRUNNEA. 3, CYPHELLA CARICINA



MISCELLANEOUS FUNGI

twisted lumen-contents projecting above the basidia as narrow hair-like structures (PLATE 55, FIG. 6).

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EXPLANATION OF PLATES

PLATE 54

Fig. 1, 2, *Lepiota brunnea* Farlow & Burt. Somewhat reduced; 3, *Cyphella caricina* Peck. × 2.

PLATE 55

Fig. 4, Structure of *Corticium vagum* showing the few substratal hyphae producing occasional erect branches that develop a more compact basidial layer, × 360; 5, *Corticium fenestratum*. Hyphae, basidia, and spores, × 360; 6, *Peniophora dissoluta*. Vertical section through the very thin sporophore, showing the basidial layer, spores, and cystidia, the latter thick-walled and with a lumen of uniform diameter (from lactic acid mount), × 360; 7, Conidiophore and spores of *Diplocladium penicillioides*, × 375; 8, *Peniophora delectans*. Vertical section through the very thin sporophore, showing the basidial layer, spores and cystidia, the latter incrusted at the tips in two cases, the third with incrustation dissolved off and showing the abruptly enlarged lumen at the apex, × 360; 9, *Cornularia macrospora*. Habit sketch of the columnar pycnidia on branch of *Robinia*, × 3; 10, *Corticium botryoideum*. Hyphae, basidia, and spores, × 360; 11, *Corticium subcoronatum*. Hyphae, basidia, and spores, × 360; 12, *Leptothyrium Periclymeni*. Section through pycnidium, and spores, × 175; 13, *Cornularia macrospora*. Single columnar pycnidium and the septate spores, × 250; 14, *Ocellaria aurea*. Section through apothicium, × 100; 15, *Ocellaria aurea*. Ascus, spores, and paraphyses, × 200.

LIFE HISTORY OF A CERCOSPORA LEAF SPOT FUNGUS OF COWPEA

DENNIS H. LATHAM [1]

(WITH PLATE 56 AND 2 TEXT FIGURES)

Among the diseases of cowpea, *Vigna sinensis* (L.) Endl. in North Carolina is one which appears as lesions on the leaves and stems. This disease has been known for at least forty years, as it was reported from Mississippi as early as 1891. It has now been under observation by the writer since September, 1931, and it is the purpose of this paper to report certain results obtained from a study of the life history of the causal organism.

SUSCEPTS

Although the writer has observed this disease on *Vigna sinensis* only, the causal fungus, *Cercospora cruenta*, Sacc., is apparently not limited to this suscept. A considerable number of other plants have been listed by various collectors as suscepts. Specimens on the following plants have been deposited in the Herbarium of Mycological and Pathological Collections, Bureau of Plant Industry. *Colopogonium sp.*, *Dolichos sp.*, *D. sesquipedalis*, *D. sinensis*, *Phaseolus sp.*, *P. aureus*, *P. vulgaris*, *Vigna Catjang*, *V. sinensis*, and *V. unguiculata*.

THE DISEASE

Range:

This leafspot disease is apparently widely distributed. According to the files of the Plant Disease Survey, *Cercospora cruenta* has been reported as occurring on *Vigna sinensis* from Alabama, Ar-

[1] From a thesis submitted in partial fulfillment of the degree of Master of Arts in the Graduate School of Duke University.

The writer wishes to express his appreciation to Dr. F. A. Wolf, under whose guidance the work was done and manuscript prepared; also to Dr. S. G. Lehman, of the North Carolina State College, for suggestions in preparing this manuscript.

kansas, Delaware, Florida, Georgia, Illinois, Indiana, Kansas, Maryland, Mississippi, New Jersey, North Carolina, South Carolina, Texas and Virginia. It has been reported also from Sumatra in the Dutch East Indies.

Symptoms:

The disease is most frequently seen on the leaves, but may occur also on the stems. On leaves, reddish brown spots appear as the most prominent early symptom. Later the tissue in these spots becomes necrotic. In size, the diseased areas may vary from a few millimeters to a centimeter or more in diameter. The outline may be rather regular to very irregular. Text figure 1 shows a leaf and an extra leaflet from naturally infected plants in the field. When infection is more severe the spots sometimes coalesce, forming large necrotic areas as is shown in figure 1. In figure 2, leaflets *a*, *b*, and *d* were covered with a grayish-black coating of conidiophores and conidia. Leaflet *c* shows definite lesions at the places where inoculations were made with a watery suspension of conidia taken from an old diseased leaf.

When conidia are being formed, the lesions are usually irregular in outline and reddish brown in color, when viewed from the upper surface of the leaflet. On the lower surface of the leaflet, the lesions are irregular in outline and reddish brown in color at first, but when the pathogen is fruiting abundantly they are dark gray to black, due to the presence of numerous conidiophores and conidia.

THE CAUSAL ORGANISM

Pathogenesis:

It is not known whether the pathogen gains entrance to the suscept by direct penetration of the epidermal walls or through the stomata. Once the germ tube has entered, the fungus develops rather slowly, and the mycelium is, at first, intercellular. The pathogen absorbs at least a part of its nutriment from the surrounding cells by means of haustoria (PLATE 56, FIG. 1). The early stage of the development of the pathogen produces a plesionecrotic condition and when the attacked cells have reached the holonecrotic condition, the mycelium may then become intracellular. Then both inter- and intracellular mycelium may be found

in the same lesion. Plate 56, figure 2, shows intercellular mycelium completely surrounding several cells.

Morphology:

After the pathogen has become established in the suscept, development preparatory to asexual reproduction begins. A single hypha branches and rebranches to form a loosely interwoven, intercellular stroma (PLATE 56, FIG. 3 and 4). The stromata usually develop in the substomatal cavities but sometimes at other places. From the stromata arise erect, dilutely olive, loosely fasciculate branches, the conidiophores. They are usually simple but may be forked or somewhat subdenticulate (PLATE 56, FIG. 5). The number and length of the conidiophores varies, apparently, with conditions under which they grow. If there is an abundance of moisture, the fungus seems to grow more luxuriantly and to produce more and longer conidiophores and conidia than when it is growing under comparatively dry conditions.

The conidia which this fungus produces (PLATE 56, FIG. 11) are acicular-obclavate, slightly curved, acute above, and hyaline becoming olive. Measurements of over one hundred conidia taken from lesions on living leaves show that they range from $35–154 \times 3.5–4.5\ \mu$. The average size of the conidia is $62.5 \times 3.7\ \mu$, with 5 septa, which very closely approximates the size given by Saccardo (7) for *Cercospora cruenta* Sacc.

Spermogonial stage:

During the latter part of September small, punctiform, fruit bodies (PLATE 56, FIG. 8) resembling pycnidia were present in old lesions and were observed to be discharging large numbers of pycnospore-like bodies having the appearance of spermatia. Since no spore forms other than conidia had previously been reported for *Cercospora cruenta*, some of the diseased leaf and stem material was collected and stored in a wire cage out of doors, in order to trace the development of the pathogen during the winter. Spermogonia were present at all times and were actively discharging spermatia, when observed at intervals, between the latter part of September, 1931, and June 1, 1932.

The spermogonia develop within subepidermal stromata. Apparently these stromata may or may not have previously given rise to conidiophores, yet spermogonia have been observed bearing

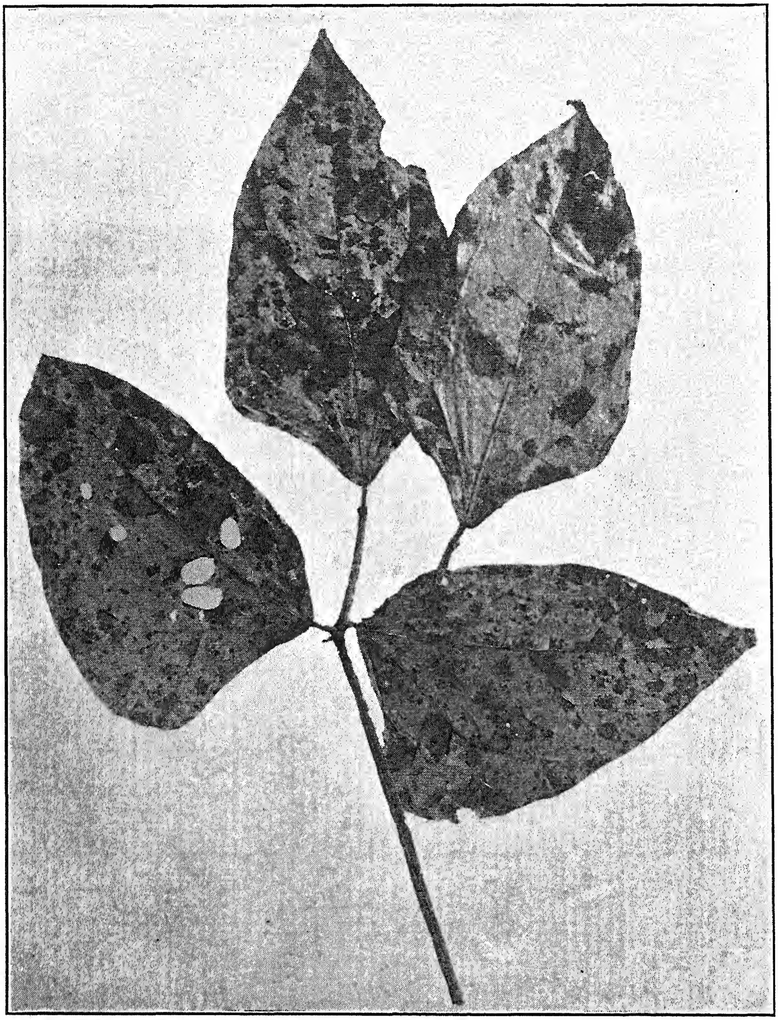


FIG. 1. Leaf of cowpea infected with the fungus.

conidiophore bases on their exposed surfaces (PLATE 56, FIG. 6). The mature spermogonia measure 31–77 $\times$ 24–70 μ. The mature spermatia are hyaline and rod-shaped and measure 2–2.5 $\times$ .8 μ.

All attempts to germinate them in tap water have failed. This is in accord with the findings of other investigators who have worked with similar stages of other species of *Cercospora* (4, 5, and 6).

Perithecial stage:

Examinations of the stored material were made at intervals of two weeks throughout the winter and spring. Early in January structures that were interpreted to be perithecial initials were observed. The perithecia develop from old subepidermal stromata and become differentiated into an inner pseudoparenchymatous medullary portion, surrounded by an outer layer or rind of a thickness of one to two brownish, thick-walled cells. The medullary tissues disappear as the asci develop. Mature perithecia are slightly beaked and measure 52–70 $\times$ 63–87 μ.

The cytological development of this species has not been fully investigated, but sectioned materials show that it is similar to that described by Higgins (4) for *Mycosphaerella Bolleana* Hig. and for *M. cerasella* Aderh. by Jenkins (6). All the asci in the same perithecium do not mature at one and the same time. One may find very young asci, in which the spores have not yet been delimited, and others which are fully matured and contain eight mature ascospores. The oldest asci are formed at the center of the perithecium and the youngest toward the lateral walls. Mature ascospores were not found before April 1. The asci are cylindric-clavate, 35–52 $\times$ 7–10 μ, bitunicate, the inner membrane excentrically papilliate, and eight-spored, the ascospores are usually arranged in two rows until the elongation of the inner ascus membrane takes place.

While examining perithecia in hanging drops of water, under the microscope, ascospores were seen to be forcibly discharged. In a number of cases the perithecia were so oriented that the tips of the asci could easily be seen when they had elongated and extended through the ostiole. Some of the asci extended as far as thirty microns beyond the orifice of the perithecium. In order to determine how the asci elongate, a perithecium was dissected from the leaf material and mounted in a drop of water on a glass slide with a cover glass over it. The perithecium was then observed under the low power and was crushed by pressing gently against

the cover glass with a dissecting needle. When a perithecium, containing mature asci, is carefully crushed, the asci are extruded in a group. With such a group of asci, one can observe the entire process of spore discharge which takes place about as follows. When the ascus is ready to discharge its spores the outer membrane breaks along one side near the apex (PLATE 56, FIG. 9 AND 10), and the inner membrane quickly elongates to 2 or 3 times its original length. By the time the inner membrane of the ascus is fully extended there is usually a single spore at its apex. Within a few seconds the remaining seven ascospores move toward the apex of the ascus. The spore at the apex is forced against the wall and soon penetrates at a point slightly to one side of the apex. The ascospores may be ejected through the water as far as fifty microns. Discharge of the first spore may be followed, singly and in rapid succession by the remaining seven spores, but there may be an interval of several minutes between discharges, or apparently sometimes the membrane is broken in another place and the seven spores are discharged in a group. The method first described is the one usually observed. As the spores are being discharged through the apical pore they are usually halted momentarily as the slight constriction at the septum comes into the opening in the ascus membrane (PLATE 56, FIG. 10). When an ascus has discharged its spores and is retracted it collapses or it may collapse without being retracted. Another ascus is then extended through the ostiole to discharge its spores in the same way. Usually more than one ascus at a time protrudes through the ostiole to discharge its spores; as one is retracted or collapsed it may be replaced by another until all the mature asci have ejected their spores.

The nature of the force that brings about the elongation of the asci and the discharge of the ascospores is not clearly understood. It was suggested by Walker and Andersen (3) that there is a considerable quantity of glycogen stored in the asci. Usually the glycogen is confined to the region below the spores, and exerts little or no osmotic pressure, but is capable of becoming rapidly transformed into sugars of high osmotic value. Such a transformation and the consequent absorption of water may be responsible for the bursting of the asci and the violent ejection of their spores. This seems to be a reasonable explanation for the violent discharge

of ascospores, especially where the spores come out in a jet. When the spores are discharged singly, as in the fungus under consideration, one must also look for an explanation of the mechanism by which the pressure within the ascus is increased, after a spore is

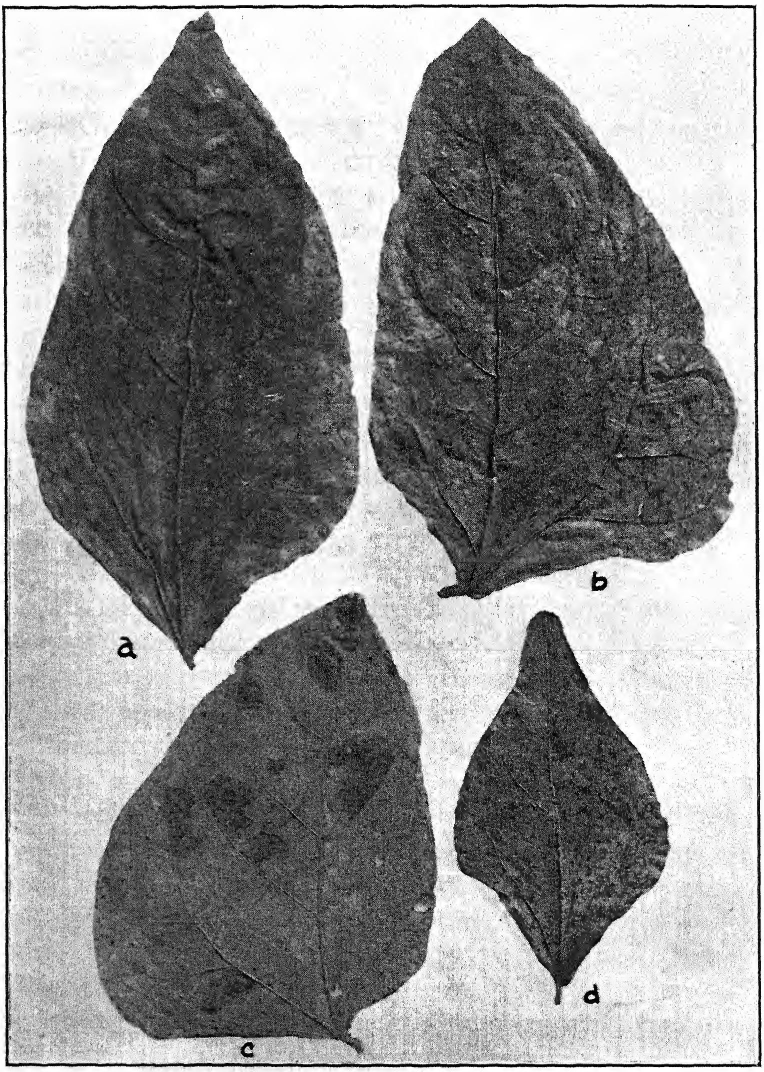


FIG. 2. Infected leaflets covered with a coating of conidiophores and conidia.

discharged, to such an extent that a second one is forced out, and so on until the eight spores have been discharged seriatim. The inner membrane, which may elongate to 2 or 3 times the length of the outer membrane, must be quite elastic. It is thought that the pore in the ascus tip closes quickly and tightly when a spore passes through it, due to the elasticity of the membrane, and that the osmotic pressure is again increased sufficiently to cause the discharge of another spore due to the transformation of more glycogen into sugar and to the absorption of more water.

When the ascospores are mature they are hyaline, unequally two-celled , and measure 11.0–19.2 × 3.5 μ, as determined by measurement of twenty-six ascospores. Only two were found to be less than 14.0 μ and only one more than 17.5 μ, in length. These three were 11.0, 13.0, and 19.2 μ respectively. Twenty-three of the twenty-six ranged from 14.0–17.5 μ, with over half of the total number measuring 17.5 μ. The ascospores germinate (PLATE 56, FIG. 7), by germ tubes growing directly out of the ends of the spore.

Isolation and culture:

The fungus has been cultured on bean agar, a decoction of cowpea leaves with agar, pieces of cowpea stem in test tubes with enough agar in the bottom of the tube to hold the stem in place and to furnish moisture, plain agar, plain agar with 2 per cent of dextrose, and potato agar with 2 per cent of dextrose. The fungus grew well on all media except plain agar. Potato dextrose agar seemed to be the most favorable one and was selected as the medium on which to study the fungus in culture.

A pure culture of this fungus, originating from a single conidium, if allowed to grow undisturbed for about four weeks on potato dextrose agar attains a diameter of approximately two centimeters. When growth first becomes visible to the unaided eye, the cultures are whitish, but as growth proceeds the mycelium becomes light gray, and by the end of two weeks has become dark gray to black. The center of the colony is usually slightly raised.

Species of *Cercospora* are regarded as being unable to produce conidia in artificial culture, except in the case of a few species. The writer used a modification of the method outlined by Ezekiel

(2) to obtain single spore cultures of the cowpea leaf spot fungus. These cultures were allowed to grow for about a week before being examined for the presence of conidia. Some of these cultures were subsequently examined every three or four days until they were about six weeks old, but no conidia were ever found. It was then decided to examine very young cultures in the search for conidia.[2] Accordingly, the writer made other single spore isolations, and found that conidia began to appear after about thirty-six hours and were rather numerous after about forty-eight to seventy-two hours. Conidia were scarcely ever found in cultures over five days old, and never occurred in cultures over ten days old. These conidia (PLATE 56, FIG. 12) appeared normal in every way and germinated in tap water. Likewise pure cultures originating from ascospores have given rise to typical *Cercospora* conidia, several of which are shown in plate 56, figure 13.

Pathogenicity:

Inoculation experiments have been conducted in the greenhouse to determine the pathogenicity of the fungus. On March 9, 1932 the tip leaflet on each of five leaves on a mature plant were inoculated with a watery suspension of conidia, obtained from a diseased plant growing in the greenhouse. These inoculations were made by placing, with a small pipette, drops of the inoculum on the leaflets. On April 11, a very definite *Cercospora* lesion was noted on one of the leaflets and a spot that appeared to be a *Cercospora* lesion was developing on another leaflet. On May 15, all the leaflets that had been inoculated showed definite *Cercospora* lesions, while the uninoculated leaflets remained healthy. On May 30, the tip leaflets on each of six leaves were inoculated with ascospores taken from old stems that had been out doors all winter. This inoculum was prepared by isolating single perithecia, washing them in changes of distilled water to remove bacteria or any conidia that might be present, mounting them in small drops of water on cover glasses, and examining with the microscope. The examinations were made by placing the cover glass over the depression in a hollow-ground slide. Each drop of inoculum thus prepared con-

[2] Suggested by Mr. Clatus M. Nagel, Department of Botany, Iowa State College, Ames, Iowa.

tained twenty-five or more mature ascospores. Infection was not evident when the plants were examined on June 12, but by June 20, lesions had formed and were covered with a felt of conidia. This fact together with the data previously given seem to constitute conclusive evidence that the ascomycetous stage and the conidial stage are genetically connected.

Briefly stated, the evidence of the genetic relationship of the conidial, the spermogonial, and the perithecial stages of the fungus here considered is as follows:

1. By observing leaf and stem material periodically from September until the following June it was found that conidia, spermogonia, and perithecia developed successively in the same lesions.

2. Spermogonial and perithecial initials have been observed to arise, from or in the old conidial stromata (PLATE 56, FIG. 6).

3. Typical *Cercospora* conidia have been found in artificial cultures (PLATE 56, FIG. 12 AND 13) isolated from conidia or from ascospores of this fungus.

4. The symptoms produced when leaves were inoculated with conidia were identical with those produced on other leaves inoculated with ascospores.

The characteristics of this fungus, in its ascigerous stage, are like those of the genus *Mycosphaerella*. Since no *Mycosphaerella* stage of *Cercospora cruenta* Sacc. has previously been described, the writer herein proposes the following new combination with brief description.

Mycosphaerella cruenta (Sacc.) comb. nov.

Cercospora cruenta Sacc. Michelia **2**: 149, 1880.

Perithecial stage:

Perithecia scattered or slightly aggregate, amphigenous but mostly hypophyllus, innate but erumpent at maturity, globose, black, ostiole only slightly papillate, $52–70 \times 63–87\ \mu$; asci fasciculate, cylindric-clavate, aparaphysate, bitunicate, excentrically papillate at apex of inner membrane, eight spored, $35–52 \times 7–11\ \mu$; the ascospores unequally bicellular, the upper cell somewhat larger, very slightly curved, hyaline, $11–19.2 \times 3.5\ \mu$ (mostly $14.0–17.5 \times 3.5\ \mu$), on decaying leaves and stems of *Vigna sp.*

Spermogonial stage:

Spermogonia scattered in and near lesions produced by the conidial stage, globular-flask shaped, black, sub-epidermal at first, later erumpent, ostiolate, 31–77 × 24–70 μ; spermatia rod-shaped, hyaline 2–2.5 × .8 μ.

Conidial stage: Cercospora cruenta Sacc.

Lesions circular to very irregular, often coalescent, varying in size up to 2 cm. or more, reddish brown; conidiophores amphigenous, arising usually from substomatal stromata, loosely fasciculate, usually simple but may be forked, somewhat subdenticulate and dilutely olive; conidia acicular-obclavate, slightly curved, may be 35–154 × 3.5–4.5 μ, and 1–8 septate, hyaline becoming olivaceous.

On living leaves and stems of *Vigna sps. Dolichos sps.* and *Phaseolus sps.*

Peritheciis sparsis vel subgregariis, hypophylis rarissime amphigenis, innatis, saepe maturitate erumpentibus, globosis, atris, ostiolo minuto perforantis, 52–70 × 63–87 μ: ascis fasciculatis, oblongo-clavatis, non-paraphysatis, bi-tunicatis, ascostomate aliquantillo a latere, octosporis, 35–52 × 7–11 μ; sporidiis distichis, bicellularibus, cellula superiore leniter latiore vix curvatis, hyalinis, 11–19 × 3.5 μ, plerumque 14–17.5 × 3.5 μ.

Hab. in emortuis foliis acque ramis *Vignae sp.*

Spermogonis autumno efformantis, globosis, nigris, immersis, deinde emergentibus, punctiformibus, 31–77 × 24–70 μ; spermatis cylindricis, hyalinis, 2–2.5 × 0.8 μ, Hab. in folliis dejectis.

Status conidicus: Maculis orbicularibus v. irregularibus, amphigenis, confluentibus, magnis, rubro-ferrugineis; hyphis fertilibus amphigenis, a stromate orientibus, laxe fasciculatis, simplicibus rare ramosis, sursum subidenticulatis, dilute olivaceis; conidiis aciculari-obclavatis, rectis v. leniter curvulis, 1–8-septatis, dilute olivaceis, 35–154 × 3.5–4.5 μ.

Hab. in folliis acque ramis vivis *Vignae sp., Phaseoli sp.* acque *Dolichi sp.*

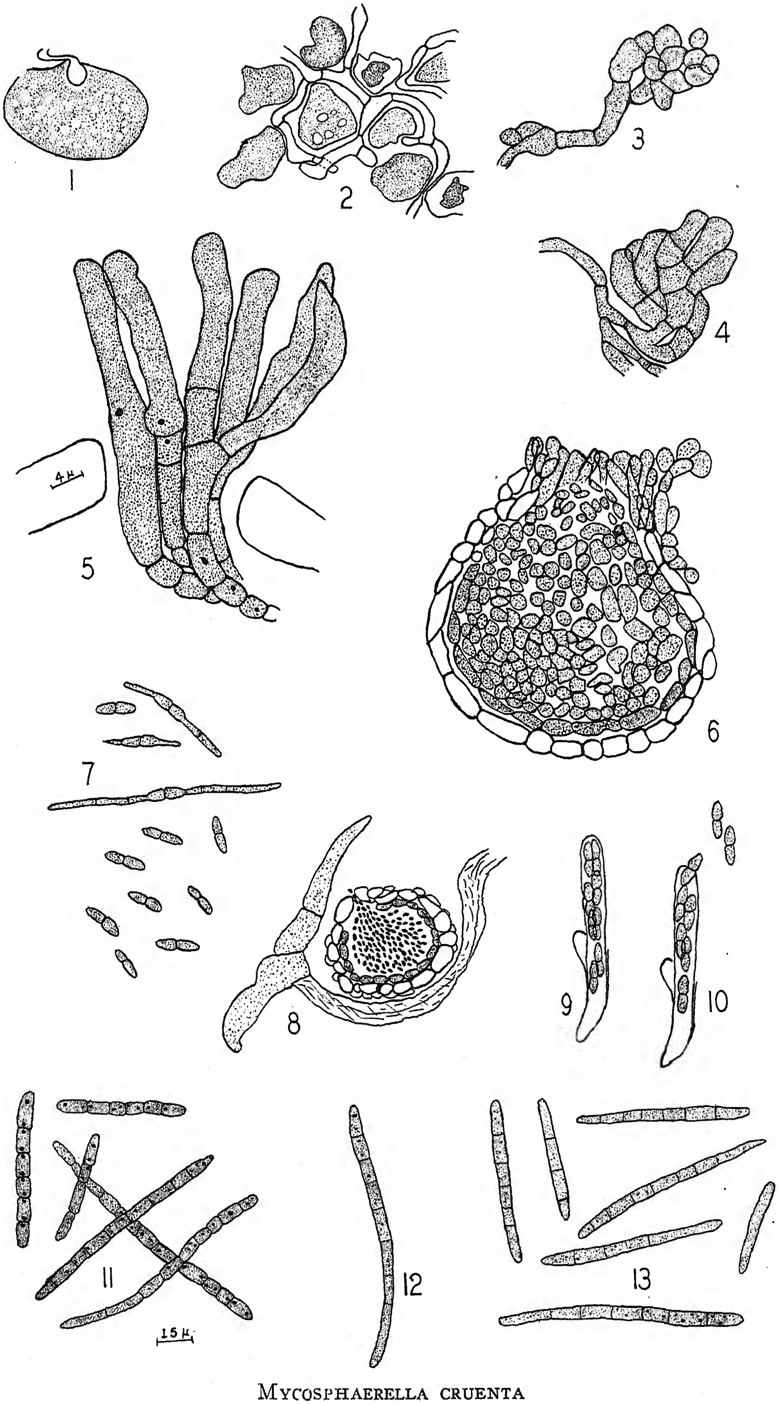
SUMMARY

This report deals with a fungus, here designated by the new combination *Mycosphaerella cruenta,* that causes a leaf and stem spot disease of cowpeas.

Lesions on the leaves are usually irregular and reddish brown. Those on the stems have not been observed to bear conidia.

The fungus has three spore forms in its life cycle. Two of these are herein described for the first time.

This disease has previously been attributed to the conidial stage,



MYCOSPHAERELLA CRUENTA

Cercospora cruenta Sacc., but evidence of the genetic connection between this conidial stage and its newly found spermogonial and perithecial stages is given.

The pathogen has been isolated in pure culture from conidia and from ascospores, both of which gave rise to typical *Cercospora* conidia.

The pathogenicity of this fungus has been established by inoculation experiments with conidia and with ascospores.

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EXPLANATION OF PLATE 56

Fig. 1, a single host cell penetrated by a haustorium; 2, a group of host cells surrounded by mycelium; 3 and 4, a single hypha showing how it branches and rebranches to form a stroma; 5, a fascicle of conidiophores protruding through a stoma; 6, an immature spermogonium; 7, a group of ascospores; 8, a mature spermagonium producing spermatia; 9 and 10, asci showing elongation of inner membrane and discharge of ascospores; 11, conidia from lesions on cowpea leaves; 12, a single conidium produced in artificial culture originating from a single conidium; 13, a group of conidia from a culture originating from a single ascospore.

A SAPROPHYTIC SPECIES OF CATENARIA ISOLATED FROM ROOTS OF PANICUM VARIEGATUM

John S. Karling

(with plates 57 and 58 and 3 text figures)

In the course of attempts to determine the degree of parasitism and host range of *Cladochytrium replicatum* Karling during the summer of 1933 a large chytrid was encountered in dead roots of *Panicum variegatum* which had been placed in a battery jar containing young growing plants of *Eriocaulon septangulare*. The large size and extensive ramifications of many thalli among the host cells were strikingly different from those of any other chytrid the author had so far observed and led to the belief at first that this species represented perhaps a new group of chytrids, but a more intensive study of its structure and development in a wide range of host plants has shown that it is undoubtedly a species of *Catenaria* which is very ubiquitous in distribution and structure. Species of this genus may infect a wide variety of plant and animal tissues, and, as has recently been shown by J. B. Butler and Humphries (1932), can readily be grown on artificial media. Under such conditions the thalli become extensively developed and are markedly variable in size and structure. The species which occurred in sterile roots of *Panicum variegatum* is strikingly similar to *C. Anguillulae* when grown on artificial media, and for this reason it is premature to give a specific name before a more extensive study has been completed.

HOST RANGE

The fact that *Catenaria* sp. occurred so abundantly as a saprophyte in roots of *Panicum variegatum* suggested that it might be cultured in other sterilized tissues as well, and a study was thus begun of its host range. It was soon found to be very ubiquitous in this respect and could be grown on a wide variety of plant and animal tissues, including nematodes, insects, algae, liverworts,

mosses, water ferns and flowering plants. In the following table are given the tissues which have been tested to date and the

TABLE 1

SHOWING THE VARIOUS STERILIZED PLANT AND ANIMAL TISSUES INFECTED WITH *Catenaria sp.*

Plants

Family	Species	Parts infected
Vaucheriaceae......	*Vaucheria* sp.	
Oedogoniaceae......	*Oedogonium* sp.	
Zygnemaceae.......	*Spirogyra crassa*	
Characeae..........	*Nitella flexilis*	Internodes, nodes and rhizoids
	N. glomerulifera	"
	Chara coronata	"
	C. zeylanica	"
	C. delicatula	"
	C. contraria	"
Ricciaceae..........	*Riccia fluitans*	Thallus, ventral scales
Marchantiaceae.....	*Marchantia polymorpha*	"
Anthocerotaceae.....	*Antoceros* sp.	Thallus
Sphagnaceae	*Sphagnum* sp.	Rhizoids
Filicales	*Osmunda regalis*	Gametophytes
Isoetaceae	*Isoetes lacustris*	Roots and fleshy parts of sporophylls
Selaginellaceae......	*Selaginella erythropus*	Roots
	S. elegans	"
	S. emiliana	"
Marsileaceae	*Marsilea quadrifolia*	"
	Pilularia sp.	"
Ceratophyllaceae	*Ceratophyllum* sp.	Fleshy leaves
Ranunculaceae......	*Ranunculus* sp.	Roots
Commelinaceae.....	*Tradescantia virginica*	Fleshy soft stems and roots
Liliaceae...........	*Hemerocallis fulva*	Roots
	Allium Cepa	"
	Lilium canadense	"
Iridaceae...........	*Iris* sp.	"
Gramineae..........	*Hordeum* sp.	Roots, scutellum, and bases of young leaves
	Zea Mays	"
	Avena sativa	"
	Triticum vulgare	"
	Lolium sp.	"
	Panicum variegatum	"
Sparganaceae.......	*Sparganium* sp.	Roots
	Alisma plantago	"
	Sagittaria lorata	"
	S. longirostra	"

Animals

Rotifers............	Various species	Eggs
Infusoria...........	Various species	Cysts
Insects.............	*Drosophila melanogaster*	Eggs

results obtained. In all tests the tissues were autoclaved in a beaker for approximately twenty minutes to insure sterility and then placed in beakers containing infected material. It is to be

noted in this table that rhizoids, roots, underground parts, and the more meristematic tissues are the principal tissues infected.

The most favorable tissue so far found for study of the structure and development of the thallus are dead onion roots. Within 12 hours the epidermal and sub-epidermal cells become heavily infected, and thalli in all stages of development may be found in great abundance. In such roots which have been boiled for approximately 10 minutes the central region and the greater part of the cortex separate readily in a day or two from the epidermal and subepidermal cells, and may be easily squeezed out at the cell end, leaving the latter in the form of an empty cylinder. Then by slitting the epidermis open and spreading it out flat on a slide, the chytrid may be seen and studied with remarkable ease and clearness. As many as 600 thalli in different stages of development have been counted in a 7 mm. long bit of such tissue.

Catenaria Anguillulae also has been reported by Sorokin (1876), Dangeard (1885, 1886), Constantineanu (1901), J. B. Butler and Buckley (1927), E. J. Butler (1928), Buckley and Clapham (1929), and J. B. Butler and Humphries (1932) from a wide range of host tissues, including eelworms, liverfluke ova, rotifer eggs, infusorial cysts, algae, and artificial media. However, no extensive study of its host range has yet been made, but the diversity of tissue on which it has been reported suggests at least that it may be very widespread. The fact that the species isolated from *Panicum variegatum* occurs also on eelworms, rotifer eggs, infusorial cysts and algae indicates a close relationship if not exact identity.

STRUCTURE AND BEHAVIOR OF THE ZOÖSPORES

The zoöspores of *Catenaria* sp. are produced in great abundance and thus offer exceptional opportunity for study of the problems of structure, germination and fusion. In many mounts of onion root tissue the number of swarmspores has been so great that the surrounding medium was a seething mass of motile darting organisms. The zoöspores are somewhat elongated and oval, and the majority vary from 4 to 5 $\times$ 6 to 7.5 μ with a single cilium attached at the posterior end. As is shown in figures 1 to 10 the cilia are approximately four to five times the diameter of the

zoöspore body in length. As they escape from the sporangium the zoöspores are usually somewhat elongated (FIG. 5), and retain this shape in the active swimming stage, but towards the end

FIG. 1. Showing probable stages in the fusion of zoöspores and the subsequent behaviour of the zygote.

of the motile period become almost spherical. Under certain conditions they may become distinctly amoeboid in shape and movement (FIG. 6 to 10).

The active swimming period lasts approximately forty minutes in tap water and under room temperature and light conditions, but may often be shortened or extended beyond this time. The swimming movement is comparatively smooth, but the zoöspores may occasionally dart about. They may come to an abrupt stop, and then dart off rapidly in another direction. So far no evidence of diplanetism has been observed. The zoöspores may become temporarily inactive when confined in a narrow space, but they have never been found to round up, retract or lose their cilia, encyst and then become active again as in the Saprolegniaceae.

As the end of the motile period approaches the swarmspores tend to assume a spherical shape and become sluggish in their movement. They may shake and whirl about actively for a few minutes without making much headway, but the movement finally dwindles down to a faint shimmer and ceases altogether. According to my

observations the cilia are not retracted but drop off and may frequently be seen lying separate in the water. The majority of zoöspores degenerate rapidly, and during this process they increase considerably in diameter and become highly vacuolated. Figures 11 and 12 respectively show late degeneration stages of bi- and uniciliated zoöspores. Within 10 to 20 minutes after becoming inactive the membrane of the zoöspore is ruptured, and the contents are disseminated in the surrounding medium.

The internal structure and contents of the zoöspore body are difficult to determine with any degree of certainty in living material. No single large, glistening and highly refractive body or globule such as occurs in most of the chytrid zoöspores is visible. One or several opaque bodies may be present in the center or slightly displaced towards the posterior end. Frequently one of the bodies may be somewhat crescentic or elongated, as is shown in figures 1, 2, 3, 5, 6, and 8. They often appear to be in a more clear space or vacuole which extends backwards to the point where the cilia are attached and seems to have some relation with it as described by E. J. Butler for *C. Anguillulae*.

The zoöspores are predominantly uniciliated, but biciliated and even triciliated forms have been observed. Figure 3 shows a triciliated zoöspore which measures 12μ in diameter. Two of the cilia are attached at one point, while the third is slightly displaced. In figure 4 is shown a biciliated individual which is almost twice the size of the normal spore illustrated in figure 1. Size differences, however, are not always correlated with the number of cilia, since, as is shown in figure 3, uniciliated zoöspores may sometimes be as large as the biciliated ones.

Two cases of fusion between zoöspores or gametes have been observed to date. Up to the present time no fusions have been described for the genus *Catenaria*, and the two cases observed in *Catenaria* sp. thus bring the genus in line with *Olpidium*, *Synchytrium*, and other chytrids in which fusion between zoöspores or motile isogametes has been reported. In text figures 1 to 7 are shown progressive stages of fusion and the subsequent behavior of the zygote. The zoöspores or gametes continued to swim about slowly as fusion progressed (TEXT FIG. 2), and finally formed a zygote such as is shown in text figures 3 and 4. The two cilia,

however, remained widely separated, and the gametes appeared to retain their individuality to a marked degree. The zygote never became perfectly round as in *Olpidium* and *Synchytrium*

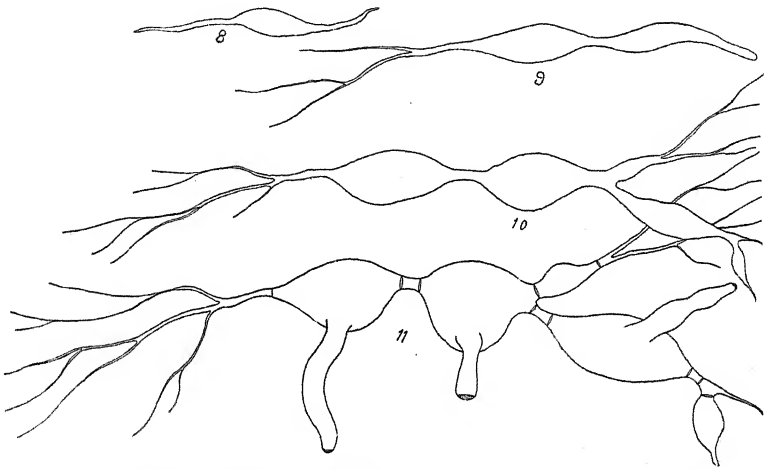

FIG. 2. Showing stages in the development of a polycentric thallus.

but remained more or less lobed. Furthermore, the denser more opaque bodies contributed by each gamete were quite distinct in size and number and their fate could readily be followed in the process of fusion. Gamete *A*, for instance, contained three bodies of different sizes, while *B*, had a single large slightly constricted one. These bodies never fused, but retained their identity as long as the zygote was kept under observation.

The subsequent behavior of the zygote was unusually interesting. Apparently the union was not entirely congenial, since both gametes made repeated attempts to separate and free themselves. Shortly after the stage shown in text figure 4 the two lobes of the zygote began to move apart, carrying their opaque bodies and cilium with them as is illustrated in text figure 5. Long pseudopods were formed in the direction of movement, and as a consequence only a narrow protoplasmic isthmus was left between the two parts. In the meantime the two cilia were very active, beating back and forth, but more or less in unison; so that they rarely became entangled. Separation of the two lobes continued further, until the zygote was stretched out over a distance of 32 μ, as is shown

in text figures 6 and 7. The connecting isthmus was drawn out to a fine strand measuring less than $\frac{1}{2}\,\mu$ in diameter, and in the process one of the cilia became stranded on the isthmus almost midway between the lobes. Failing to separate the two gametes slowly came back together, and again appeared very much like text figures 5 and 2. Separation was again attempted and repeated twice. Finally the zygote assumed the shape shown in text figure 4 and swam away. It thus became impossible to keep it in the field of observation for more than an instant, and I was unable to determine whether separation was eventually completed. Somewhat similar changes in shape and behavior were observed in the second case of fusion also.

In light of Cotner's (1930) studies on the development of zoöspores in the Oömycetes, the presence of numerous biciliated and occasional triciliated individuals is not to be taken, it seems to me, as conclusive evidence of sexual fusion in every case. Incomplete cleavage and the formation of unusually large segments may readily lead to the development of biciliated and triciliated zoöspores without involving fusions. Zoöspores with supernumerary cilia have been observed and described in many fungi in which no fusion of motile gametes has been seen. In *Physoderma Zeae-Maydis,* for instance, Miss Ojerholm (1934) reports that approximately one per cent of the swarmspores are biciliated, and yet few unmistakable cases of fusion could be found. The literature in relation to supernumerary cilia and fusion of motile isogametes among the chytrids has been adequately summarized by Miss Ojerholm.

DEVELOPMENT OF THE THALLUS

The majority of zoöspores degenerate shortly after coming to rest, and so far only two cases of germination have been directly observed. However, the epidermal cells of onion roots are frequently filled with small thalli, which indicates that a large proportion germinate. In figure 13 are shown two of the germination stages observed. At the left is a zoöspore lying on the host cell wall, and on the right is one which has partially penetrated. Complete entrance of this individual was not observed, so that it is impossible to say whether or not its entire content moved

bodily through the host cell wall. The stage here shown is quite different from that figured by Dangeard and J. B. Butler and Humphries for *C. Anguillulae* outside of the host cell and on artificial media; but strikingly similar to J. B. Butler's and Buckley's figures of germination on the ova of the liverfluke.

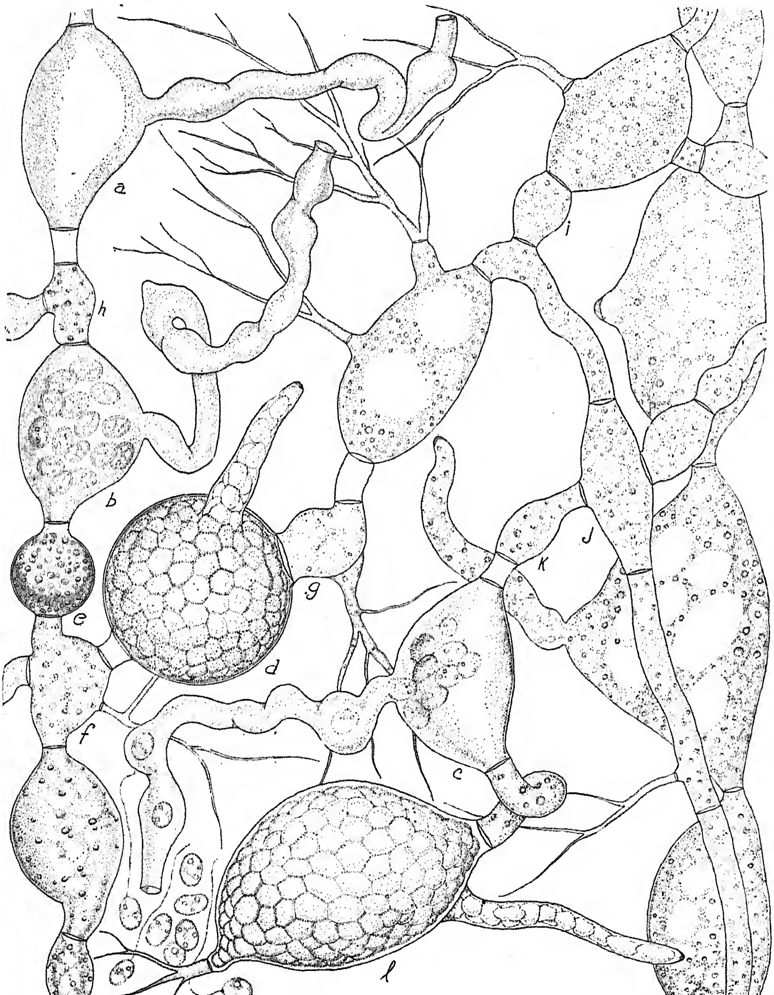

FIG. 3. A large extensively branched thallus elongated to a distance of 3.2 mm. in the degenerating pith of *Panicum variegatum*; *a, b, c,* zoösporangia with greatly elongated necks; *d* and *e,* zoösporangia with cleavage segments; *f, g, h, i, j,* and *k,* enlargements on the thallus apart from the zoösporangia.

The thallus of this species, like *C. Anguillulae,* exhibits without doubt the greatest range of variation in size, shape and organization of any known chytrid. The thalli may be unicellular, spherical, globular, elongated, and irregular without any trace of rhizoids (FIG. 14, 15, 22, 23) like species of the Olpidiaceae; monocentric with one to several rhizoids (FIG. 17, 18, 19, 24) as in the Rhizidiaceae, or multicellular, extensive, polycentric in organization and definitely mycelium-like (FIG. 20, 25, 26, 32) as in members of the Cladochytriaceae and higher fungi. It thus shows all the various types of organization that are found in the whole order of chytrids (Karling, 1932), but the multicellular polycentric habit of growth and development is predominant. All of these types of thalli have been found in onion roots, so that they are not to be regarded as the result of differences in the particular host in which they are growing. The general tendency, however, is for the thalli to be more rounded, condensed and less extensive in insect eggs, and more mycelium-like and extended in the long internodal cells of the Characeae. In *C. Anguillulae* Dangeard likewise observed reduced monocentric thalli which might readily be mistaken for a species of *Olpidium,* and on this basis he questions the validity of Braun's *O. endogenum* and Nowakowski's *O. gregarium* and suggests that in some cases they may only be a form of *C. Anguillulae.*

In figures 14 and 15 are shown two young spherical thalli from epidermal cells of *Allium Cepa* in which rhizoids are completely lacking. Several such thalli of this nature have been observed to grow to maturity and produce normal zoöspores. Figure 22 shows a spherical thallus with a well developed sporangial neck. In such individuals the entire thallus at maturity is transformed into reproductive cells and thus becomes monogeocentric as in *Olpidium* and other members of the Myxochytridineae. The thallus shown in figure 23 is quite irregular and under low powers of magnification superficially resembles a large amoeba.

Early developmental stages of monocentric thalli with one to several rhizoids are shown in figures 16 to 18. The one illustrated in figure 16 was followed to maturity in a bit of onion root epidermal tissue mounted in a hanging drop chamber. The two rhizoids developed extensively and produced several branches,

while the incipient sporangium became spherical with maturity. Figure 19 shows another similar thallus with two rhizoids. The contents of the sporangium has undergone cleavage into zoöspore segments, and a well defined sporangial neck is present. The rhizoids are very thick at their point of origin on the surface of the sporangium, 2.5 μ to 7 μ, and are separated from it by cross walls. This latter characteristic is perhaps the most essential difference between the monocentric thalli of *Catenaria* sp. and those of the Rhizidiaceae. In the majority of species of this family, as far as the evidence in the literature goes, the rhizoids are continuous with the remainder of the thallus at maturity and no septa are formed. In figure 24 is shown a thallus with a highly branched system of rhizoids which extended for a distance of 320 μ among the host cells. This compares favorably in extent and size with the rhizoidal systems of *Entophlyctis* and *Diplophlyctis* (Karling, 1928, 1931) which without doubt represent the maximum of rhizoidal development in the known rhizidiaceous forms. At the point where they pass through the host cell they are usually somewhat constricted, but this depends to a large extent on the state of degeneration of the host tissue.

The incipient sporangium in monocentric as well as polycentric thalli may become extensively developed in size and shape and ramify several host cells. The sporangial portion of the thallus shown in figure 24 has budded out and entered six adjacent cells, three lying above and four beneath. These lobes were all in continuity at the time the drawing was made, but at maturity septa were formed between them, making thus seven distinct sporangia. In one case observed a single sporangium had budded out and penetrated fourteen adjacent host cells, producing a more or less cluster of rounded lobes.

The early development of polycentric thalli is not essentially different from that of monocentric ones, as far as my observations go, with the exception that they are usually more elongated. In text figures 8 to 11 are shown stages in the development of such a thallus under hanging drop condition in room light and temperature. The development here figured for *Catenaria* sp. is strikingly like that reported by Dangeard, J. B. Butler, and Buckley, E. J. Butler and J. B. Butler for *C. Anguillulae*.

A partially mature polycentric thallus with eight incipient sporangia is shown in figure 20. Cross walls have been formed between the sporangia, but the rhizoids are still continuous with them. These were likewise later delimited by septa. The sporangia in this thallus remained long and cylindrical throughout and never became spherical. In the long internodal cells of various species of *Nitella* and *Chara* the thallus became markedly mycelium-like and extended in some instances for more than 2.5 mm. Two such thalli are shown in figures 25 and 26. Cross walls in such cases are numerous throughout the entire length, and rhizoidal development is somewhat attenuated. At maturity the sterile cells between the sporangia usually become empty and play no part in reproduction. In certain regions they may be very fine and not more than 3 μ in diameter.

The most extensive thallus so far observed is partially shown in figure 3. It extended for a distance of 3.2 mm. in the soft degenerating inner cortex of a *Panicum variegatum* root, and had numerous long side branches. Twenty-two sporangia of various sizes and shapes were distributed at irregular intervals along the thallus together with numerous enlargements from which side branches arose. Such thalli, however, are not remarkable and unusual in the *Catenariae*. J. B. Butler and Humphries have figured a thallus of *C. Anguillulae* growing on artificial media which was composed of more than two hundred and seventy sporangia. It is not certain, however, from their drawing that all of these constitute a single thallus.

STRUCTURE, SIZE AND SHAPE OF THE SPORANGIA

The shape and size of the sporangia are so variable that it is almost impossible to give representative drawings and measurements. They may be irregular, amoeboid, oval, cylindrical and spherical in shape. The spherical sporangia at maturity vary from 12 to 64 μ in diameter, while the cylindrical ones may be less than 7 μ in width and 160 μ in length. The range of variations in size and shape is very similar to that of *C. Anguillulae* described by J. B. Butler and Buckley, E. J. Butler, and J. B. Butler and Humphries. The exit canals or necks of the sporangia are quite variable in length and diameter. When growing in the inner cortex

of roots the necks may be as much as 250 μ in length and unusually irregular as is shown in figures 31, 32*a, b,* and *c*. This irregularity in diameter appears to be largely due to the fact that where they pass through the host walls they become constricted in the same manner as the rhizoids and the remainder of the thallus. When the sporangia lie in the epidermal cells the necks are more or less uniform in diameter and often no more than 10 μ in length. Upon reaching the surface of the host tissue they usually grow a few microns beyond as is shown in figure 22*a*. At the tip or apex is a slightly denser cap which appears to be universally present but is difficult to observe clearly in living material. E. J. Butler has reported a similar thickening of the apex in *C. Anguillulae*. Whether or not there is an operculum or lid present which is pushed off as the zoöspores escape is uncertain, since I have yet failed to observe the initial opening of a sporangium.

In view of the fact that the majority of species of the family Cladochytriaceae in which *Catenaria* has generally been placed have distinct enlargements on the rhizomycelium apart from the sporangia, special attention has been given to the presence of such structures in *Catenaria* sp. These enlargements are particularly conspicuous in *Cladochytrium, Physoderma* and *Urophlyctis,* vary considerably in size and shape, and have been given a variety of descriptive names. In a previous paper (1932) the author questioned the presence of " sammelzellen " or turbinate organs in the genus *Catenaria,* and pointed out that the particular host tissues in which they have been studied were not particularly favorable for their demonstrations. The long internodal cells of *Chara* and *Nitella* allow perhaps the maximum extension of the thallus of *Catenaria* sp. without crowding, and such host plants have been employed and carefully studied for the problem of " sammelzellen " in this genus. In studying such structures, however, only mature thalli have been used, since in immature ones they are indistinguishable from incipient sporangia. Under such conditions enlargements of the thallus have been found which vary in size and shape and do not always develop into sporangia. In figure 27 is a simple spindle-shaped body from a mature thallus which was devoid of content and closely resembled the unicellular collection cells of *Cladochytrium* (Karling, 1931). Additional enlargements

are shown in figures 28, 29 and 30 and at *d, e, f,* and *g,* figure 32. The ones drawn in figures 28 and 29 have given rise to several thallus branches and thus appear to be centers of duplication. No septate enlargements as in *Cladochytrium* have so far been found, and as a general rule they look more like the swellings on the thallus of *Hyphochytrium infestans* described by Zopf (1884). However, in spite of their occasional presence in *Catenaria* sp. the author is not inclined to regard them as centers of replication as in *Urophlyctis* (Jones and Dreschler, 1920). The enlargements shown in figures 27 to 30 may possibly be nothing more than incipient zoösporangia which failed to mature. Figure 21 shows a center with five radiating branches which resembles figure 28 with the exception that it has developed into a zoösporangium and formed a long exit tube. It is to be noted in this connection that J. B. Butler and Humphries failed to find definite " sammelzellen " in *Catenaria Anguillulae,* while growing extensively on artificial media.

In all of my observations to date the zoöspores become active in the zoösporangium, but due to crowding and pressure they appear merely to glide upon one another. After part of the mass has escaped the remaining ones become increasingly active, and the cilia become visible. If the neck becomes obstructed the content of the zoösporangium becomes a churning mass of swarmspores endeavoring to escape. In all sporangia observed the zoöspores emerged one by one and immediately swam away.

SUMMARY

1. *Catenaria* sp. was isolated from sterilized roots of *Panicum variegatum* which had been placed in a battery jar containing *Eriocaulon septangulare,* and appears to be a saprophyte capable of growing on a wide variety of dead tissue. It has been grown successfully in plant and animal tissues including nematodes, insects, algae, mosses, ferns, and angiosperms.

2. The thallus may be mono- or polycentric in organization, structure and development. Unicellular, spherical, oval and cylindrical thalli without any trace of rhizoids or vegetative parts as in the Myxochytridineae are not uncommon, and at maturity are transformed wholly into reproductive cells. Monocentric thalli

with one to several rhizoids as in the Rhizidiaceae are frequently produced. The predominant type of thallus, however, is polycentric with numerous zoösporangia, rhizoids, and enlargements scattered irregularly along its length.

3. In insect eggs the thallus is usually condensed and crowded, but in soft meristematic plant tissues and the internodal cells of *Chara* and *Nitella* may become distinctly mycelium-like and extend to a length of 3.2 mm. The thallus is unicellular and continuous when young, but at maturity becomes multicellular by the formation of numerous cross walls along its length. The rhizoids are likewise separated from the remainder of the thallus by septa at maturity.

4. The zoösporangia are extremely variable in size and shape, ranging from spherical, oblong, cylindrical, amoeboid, irregular to star-shaped. Occasional enlargements occur in the thallus which may or may not develop into zoösporangia and resemble somewhat the " sammelzellen " of *Physoderma* and *Cladochytrium*. Whether or not they are to be regarded as such and occasionally serve as centers of replication is uncertain.

5. Zoöspores are produced in great abundance and vary considerably in size. They are predominantly uniciliated, but numerous biciliated and occasional triciliated ones occur. Two cases of fusions between zoöspores or gametes have been observed.

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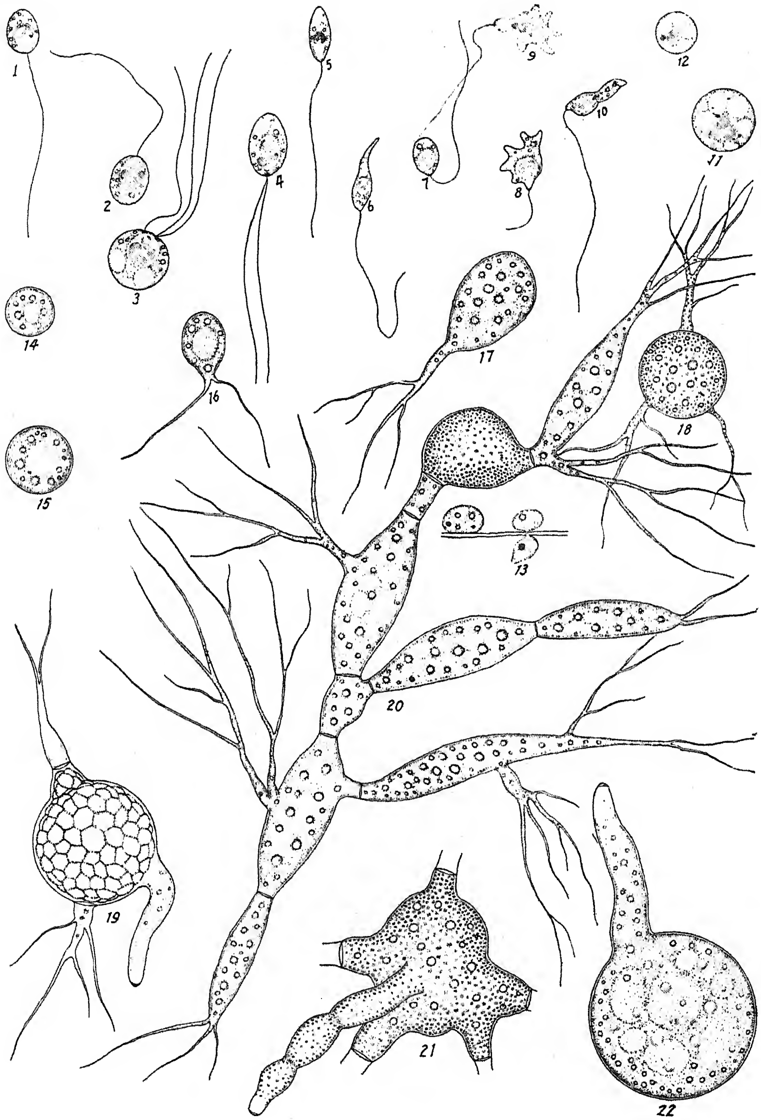
DESCRIPTION OF PLATES

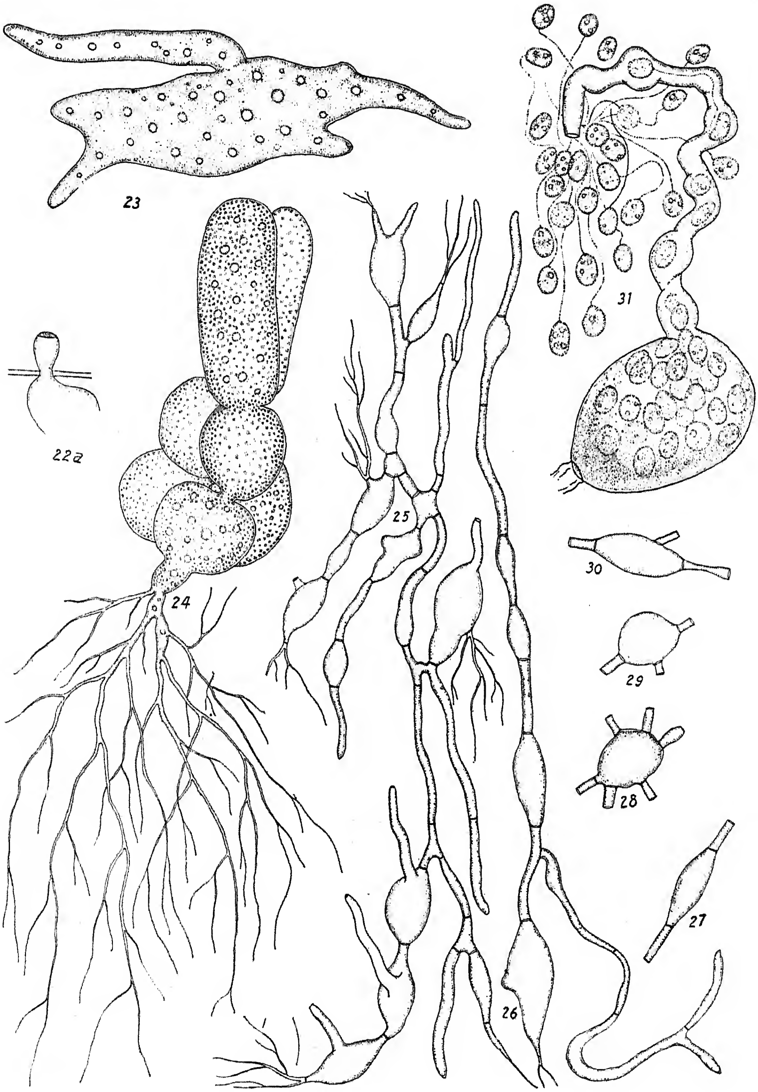
All drawings were made from living material with the aid of a Spencer camera lucida and a Zeiss 1.30 N. A. apochromatic objective and compensating oculars nos. 6 and 8. Unless otherwise indicates all drawings are from *Allium Cepa.*

PLATE 57

Fig. 1, A normal-sized uniciliated zoöspore with one large somewhat crescentric body in the center; 2, A large uniciliated zoöspore; 3, A large triciliated zoöspore measuring 12 μ in diameter; 4, A biciliated zoöspore approximately twice the size of a normal uniciliated individual; 5, Zoöspore at the time of escape from the zoösporangium; 6, Elongated zoöspore in the process of escaping from a confined area; 7, A small uniciliated zoöspore; 8, 9, Amoeboid changes in shape and movement of zoöspores in confined quarters; 10, Constricted zoöspore; 11, 12, Late degeneration stages of bi- and uniciliated zoöspores; 13, Early infection stages on epidermis of *Allium Cepa;* 14, 15, Young spherical monocentric thalli lacking rhizoids; 16, 17, Young monocentric thalli with rhizoids; 18, Small, spherical mono-



Catenaria Anguillulae



Catenaria Anguillulae

centric thallus with three rhizoids; 19, A large mature monocentric thallus with two rhizoids and a well developed neck. The content has undergone cleavage; 20, Immature, polycentric, septate thallus from pith of *Panicum variegatum* with eight incipient zoösporangia and numerous rhizoids; 21, An irregular, somewhat star-shaped zoösporangium developed at the intersection of several thallus branches; 22, A large, spherical, almost mature monocentric thallus lacking rhizoids; *22a,* Neck of a sporangium projecting slightly beyond the host cell wall and somewhat thickened at the apex.

PLATE 58

Fig. 23, An irregular, amoeboid-shaped monocentric thallus with three well developed exit tubes; 24, A large monocentric thallus with an extensive rhizoidal system. The incipient zoösporangium has budded out into six adjacent host cells; 25, An extensive mycelium-like polycentric thallus from internodal cells of *Nitella flexilis;* 26, An elongated thallus from internodal cells of *Chara coronata;* 27–30, Variously shaped, unicellular enlargements of the thallus; 31, Zoösporangium from the pith of *Panicum variegatum.* Sporangial neck twisted and irregular in shape and diameter.

THE AMERICAN AND JAPANESE MATSU-TAKES [1]

S. M. Zeller and K. Togashi

(with 6 text figures)

Along the Pacific slope of Oregon and Washington occurs a species of *Armillaria* which is assiduously collected by Japanese residents for commercial purposes. These local people have applied the name "Matsu-take" (pine mushroom) to this American species as they had applied it to one of the choice mushrooms

Fig. 1. Showing the general habitat of *Armillaria matsutake* in its association with *Pinus densiflora*, Suzukawa-mura, Prefecture Yamagata, Japan, October 16, 1932. Photo by Unya Kato.

(*Armillaria matsutake* Ito & Imai) of Japan. Many of the Japanese who reside in Oregon and collect the American Matsu-

[1] Published as Technical Paper No. 215 with the approval of the Director of the Oregon Experiment Station. Contribution from the Department of Botany in coöperation with the Department of Plant Pathology, Morioka Imperial College of Agriculture and Forestry, Morioka, Japan.

take, hold to the opinion that it is identical with the Matsu-take with which they were familiar in their native land. For a number of years we on the Pacific Coast have been interested in a more exact understanding of the taxonomy of the western species and its possible con-specific relationship with the Matsu-take of Japan. In order that we might reach such an understanding the authors have exchanged herbarium collections of the two forms from various locations, together with more or less critical notes, photographs, etc. It is the purpose of this paper to bring together notes that might be of general interest to mycologists, and to show the close relationship between the two Matsu-takes.

THE JAPANESE MATSU-TAKE

A complete discussion of the taxonomy of the Japanese *Matsu-take* has been published by Ito and Imai.[2] Since 1878 when Berkeley [3] described *Shii-take* (*Cortinellus Berkeleyanus* Ita & Imai) as *Armillaria edodes* several European, Japanese, and American taxonomists have taken the liberty to propose a binomial for either *Matsu-take* or *Shii-take*. Ito and Imai found by studying collections in European herbaria that names had been so jumbled between the two fungi that it seemed necessary to give each a new name. Matsu-take accordingly in Japan now goes under the name

ARMILLARIA MATSUTAKE Ito & Imai, Bot. Mag. (Tokyo) **39**: 327. 1925.

Fructifications gregarious or cespitose, sometimes single, large, 10–16 cm. high; *pileus* 8–20 cm. broad, thick, firm, hemispherical then expanding, convex to broadly or almost placo-umbonate; *surface* dry (to slightly subviscid in fresh condition) easily stained by clay-like soils, dull when dry, fibrous scaly, often fissured radially toward the margin, sepia tones, disk becoming tawny, russet or Mars brown,[4] fibrils concolorous with disk (drying

[2] Ito, S., and S. Imai. On the taxonomy of Shii-take and Matsu-take. Bot. Magazine (Tokyo) **39**: 319–328. *Pl. 6.* 1925.

[3] Berkeley, M. J. Enumeration of the fungi collected during the expedition of H. M. S. "Challenger," 1874–1875. Jour. Linn. Soc., Bot. **16**: 38–54. 1878.

[4] Ridgway's Color Standard was used as an aid in the descriptions.

chestnut-brown to mummy-brown or even blackish-brown in very old specimens); *margin* inrolled under the slightly persistent veil, only slightly lighter than the disk; *flesh* white to cremeous, firm

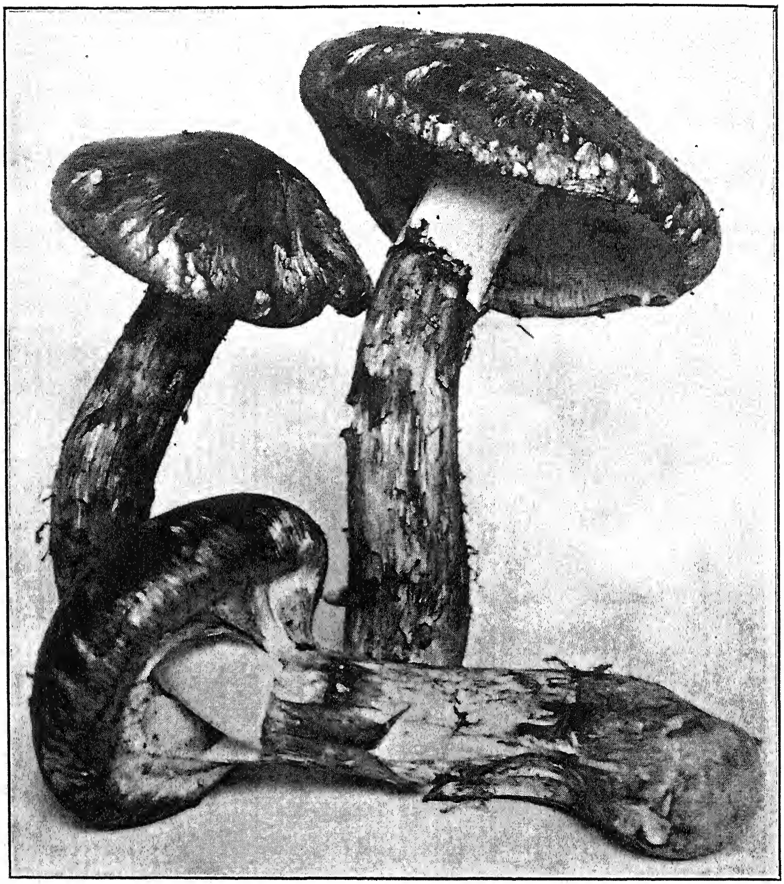


FIG. 2. *Armillaria matsutake* collected at Iwate-gun, Prefecture Iwate, Japan, October, 1932. Photo by K. Togashi. × 4/5.

with an especially agreeable (spicy) *odor* and *taste; gills* sinuate or adnexed, usually breaking free at maturity, close, 10–12 mm. broad, whitish changing buff at maturity; *spores* white, smooth, broadly ellipsoidal, 1-guttulate, 3–8 × 3–6 μ (5.57 ± 0.02 × 4.40 ± 0.01 μ), mature spores appendiculate; *stem* 10–15 cm. long, 1.5–4 cm. thick, sub-equal or tapering upward, sometimes with

enlarged base, coated by the fibrillose scaly veil and concolorous with pileus below the annulus, whitish and smooth or innately fibrillose above; *annulus* ample, persistent, membranous (at first cortinate-membranous at margins), sometimes sheathing or fluted upward, superior.

Usually in soil of the Chichibu palaeozoic type or decomposed granite. Usually associated in a mycorrhizal relationship with *Pinus densiflora* on the main island (Honshu) of Japan. September to November; sometimes August, especially in the northern districts.

Ecology of Armillaria matsutake.—According to Masui [5] the Japanese Matsu-take is an obligate mycorrhiza-former on *Pinus densiflora.* The fructifications thus may originate without any direct connection with humus in the soil but merely from an interwoven network of mycelium projecting from numerous mycorrhizas on the roots of the host. For the most part, therefore, mushroom hunters in Japan look for Matsu-take under *Pinus densiflora,* since they realize that the living roots of this tree play an essential rôle in the production of this mushroom. Masui cites cases where no mushrooms occur in certain places the year after pine trees were cut. If the main roots only are cut Matsu-take does not appear from the soil where rootlets of cut trees are distributed. Living roots of a host tree must be present for the production of Matsu-take.

It seems, however, that occasionally Matsu-take is associated with other trees. Most of the specimens cited below were collected under the Japanese Red-Pine (*Pinus densiflora*) but the specimens from South Saghalien were found under Abies sp. according to Mr. T. Ishiyama, Botanical Institute, Agricultural Department, Hokkaido Imperial University, Sapporo. Through the courtesy of Mr. Ishayama we obtained the Saghalien material collected by a ranger of the Tomarii Forestry Station. Dr. S. Kawamura [6] says Matsu-take often grows under fir trees (*Abies firma* Sieb. & Zucc.) even in the main island of Japan and the Taiwan strain

[5] Masui, K. A study of the ectotrophic mycorrhizas of woody plants. Kyoto Imp. Univ., Coll. of Sci., Memoirs (Series B) **3**: 149–279, *illus.* 1927.

[6] Kawamura, S. Explanatory diagrams of Japanese mushrooms, Tokyo, 1929. (Japanese.)

of Matsu-take from the Island of Formosa (cited below) occurs under Niitaka Red-Pine trees (*Pinus taiwanensis* Hayata) according to Sawada.[7] He describes this Formosan (Taiwan)

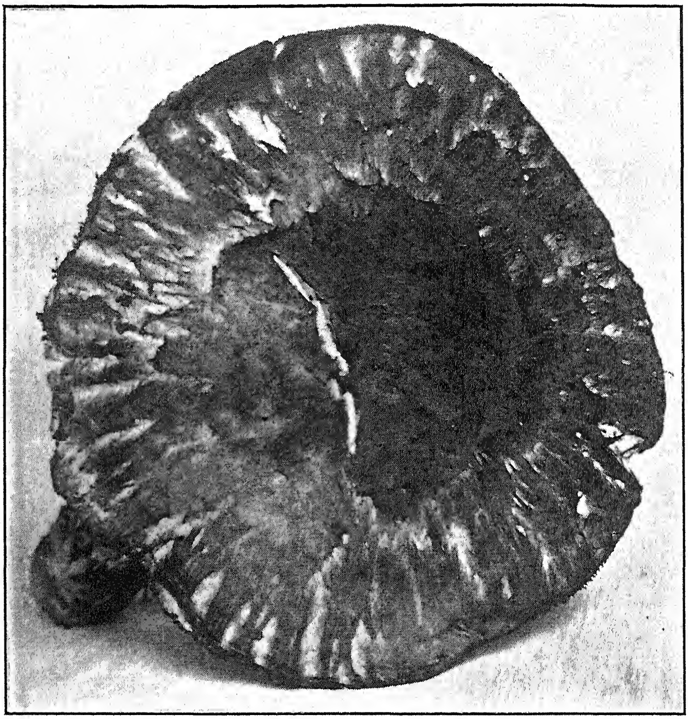


FIG. 3. *Armillaria matsutake* showing the characters of the upper surface of the pileus, collected at Kasugabe, Prefecture Hyogo, Japan, October, 1932. Photo by E. Togashi. ×4/5.

material as differing somewhat from that of the main island, *i.e.*, it has more delicate and fewer fibrils and consequently the pileus is lighter in color, the basidia are longer with only 2 sterigmata in most cases, and the spores are more oily.

In Japan several Hymenomycetes occur under the same ecological conditions and in association with the Japanese Red Pine. In the central and southern districts of Honshu *Cortinarius elatior, C. cinnamomeus, Armillaria caligata, Boletus bovinus, Cantherellus*

[7] Sawada, S. Descriptive catalog of the Formosan fungi. Part V, p. 90. 1931.

floccosus, and *Rhizopogon rubescens* are to be mentioned in this relation. *R. rubescens* develops a little later than the others, continuing its production to the next spring. While in the northern districts of Honshu *Boletus bovinus, B. elegans,* and *Armillaria caligata* are the essential associators of *A. matsutake.*

Specimens of *Armillaria matsutake* from the Japanese Empire examined by both authors:

South Saghalien: Tomarii, Sept. 20, 1932 (sent by *T. Ishiyama*).
Honshu: Pref. Iwate, Kadoma, *Kogo Togashi,* Oct. 11, 1932, Shimohei-gun, *K. Togashi,* Oct. 10, 1932 (last two collections taken at market of Morioka city); Pref. Gifu, *Makoto Hiura,* Oct. 12, 1932 (taken at market of Gifu city); Pref. Osaka, Minomo, *Yasuhiko Sato,* Oct. 23, 1932; Pref. Hyogo, Goyu, *Y. Sato,* Oct. 6, 1932, Kasugabe, *Y. Sato,* Oct. 12, 1932; Pref. Yamaguchi, Asa, *Fukundo Monde,* Oct. 23, 1932.
Formosa: Niitaka-gun, Banaiko, *Takuji Kato,* Sept. 25, 1932 (sent by *F. Onuma*).

THE AMERICAN MATSU-TAKE

The "Pine-mushroom" or "Japanese mushroom" as it is called along the Pacific Coast of Oregon and Washington was first described from this region by Murrill [8] as *Armillaria arenicola.* The late Dr. Kauffman [9] made collections at Hoodsport, Mason County, Washington, where it occurred "common enough to be assiduously collected by Japanese for commercial purposes." He said further, "I have referred this collection to *A. arenicola,* but except for the recorded accounts of the spores of *A. ponderosa,* it could be just as well referred to the latter."

Burt [10] says of *Armillaria ponderosa* that "Peck published the spores as globose, 4 μ in diameter, but they are more elongated in the Vermont specimens, determination of which was confirmed by Peck, and also in the New Brunswick (Canada) gathering" illustrated in the Icones (*pl. 13*). In the description Burt gives the spores as "white, even, 6–7 $\times$ 4–5 μ." Murrill's description

[8] Murrill, W. A. The Agaricaceae of the Pacific Coast, I. Mycologia 4: 212. 1912.

[9] Kauffman, C. H. The genus *Armillaria* in the United States, and its relationships. Mich. Acad. Sci. Papers 2: 53–67, *illus.* 1922.

[10] Farlow, W. G., & E. A. Burt. Icones Farlowianeae, *p. 15.* 1929.

of the Pacific Coast form gives "spores globose, smooth, hyaline, 4–6 μ." He also says "In general appearance, it resembles *Armillaria magnivelaris* Peck," commonly recognized as a synonym of *A. ponderosa* Peck. The collection of *A. magnivelaris* taken at Copake, N. Y., October, 1872, by Chas. H. Peck, seems identical with the western material. All of the spore measurements published for the eastern and western material can be easily included in our measurements given below for the Florence gathering in particular. This gathering of more than 200 specimens gave a very wide range in sporophore development and the range of spore measurements was quite inclusive. We feel little hesitancy in suggesting the below synonymy and adopting *Armillaria ponderosa* (Peck) Sacc. as the name of the Pacific Coast form.

The agreeable, slightly spicy odor of the western plant is difficult to describe accurately, but it is unmistakably distinctive. Some have described it as spicy, while others think it similar to the odor of sandal wood.

The synonymy of the American Matsu-take is as follows:

ARMILLARIA PONDEROSA (Peck) Sacc. Syll. Fung. **5**: 58. 1887 (*Agaricus ponderosus* Peck, Bull. Buffalo Soc. Nat. Sci. **1**: 42. 1873);—*Agaricus magnivelaris* Peck, Ann. Rept. N. Y. State Mus. **29**: 66. 1878;—*Armillaria magnivelaris* (Peck) Murrill, N. Am. Fl. **10**: 37. 1914;—*Armillaria arenicola* Murrill, Mycologia **4**: 212. 1912.

Fructification mostly single, often gregarious, sometimes cespitose, very large, 10–15 cm. high; *pileus* 8–21 cm. broad, thick, firm, convex or broadly umbonate to gibbous, then nearly plane when expanded; *surface* dry to subviscid so that sand and conifer needles adhere slightly, shining to dull when dry, glabrous or becoming fibrillose (sometimes fibrous scaly after rains), at first white or pinkish buff, disk and fibrils becoming light ochraceous-salmon (liver brown, russet, or even Mars brown *in extreme cases*), fibrils concolorous with disk; drying pale ochraceous-buff to warm buff, fibrils tawny to russet; *margin* inrolled under the slightly persistent veil, white to cremeous, usually lighter than the disk; *flesh* white, firm, with an agreeable, somewhat spicy odor and taste; *gills* emarginate, becoming sinuate-adnexed, or break-

ing free at maturity, close to crowded, 8–12 mm. broad, whitish to light buff in young specimens becoming warm buff or even ferruginous, changing liver brown where bruised; *spores* white, smooth, subglobose to oblong, 1-guttulate, $4–7 \times 3–5\ \mu$ ($5.16 \pm 0.01 \times 4.17 \pm 0.01\ \mu$) *stem* 10–15 cm. long, 2–4 cm. thick, cylindrical or tapering downward, coated by the veil and same color as pileus below the annulus, changing rubescent to liver brown where bruised or exposed, scales below annulus more or less prominent, white and furfuraceous above the annulus; *annulus* ample, persistent, membranous (at first the margins cobwebby from the cortinate-membranous veil), sometimes sheathing or fluted upward, above the middle of the stem.

Usually in sandy locations. Although described as 10–15 cm. tall the plants may push but a few centimeters above the surface of the sand, exposing usually a small portion of the cap while the stem is buried deep in the sand. Usually in rather open pine barrens, but found under several genera of conifers. October to December on the Pacific Coast of Oregon and Washington, U. S. A. Quite abundant where found.

Ecology of Armillaria ponderosa.—Along the coast for the most part this fungus is found under *Pinus contorta* but around the base of Mt. Hood it is reported under other conifers, especially *Pseudotsuga taxifolia,* under which Mr. Ethan Allen, a resident of Waldron on Waldron Island, reports it from the San Juan Islands. He says on Waldron Island it also occurs under *Pinus contorta, Tsuga heterophylla,* and *Thuja plicata,* and often among such under-growth as *Gaultheria Shallon* and *Chimaphila umbellata.* Mr. Allen says *Armillaria ponderosa* always follows in the same type of locations where *Russula delica* had occurred about two weeks earlier. In the Florence district, Lane County, Oregon, this mushroom occurs under similar conditions as do *Armillaria robusta, Amanita muscaria, Boletus edulis,* and *Tricholoma equestre,* and *Rhizopogon occidentalis* and *R. rubescens. Boletus edulis* appears several weeks earlier. During November, 1933, rhizomorphic connections have been traced between the basidiocarps and ectotropic mycorrhizas on rootlets of *Pinus contorta* at Waldport, Oregon.

Specimens examined:

New York: Columbia Co., Copake, *Chas. H. Peck,* Oct., 1872 (type of *Armillaria magnivelaris* Peck, in N. Y. State Museum).

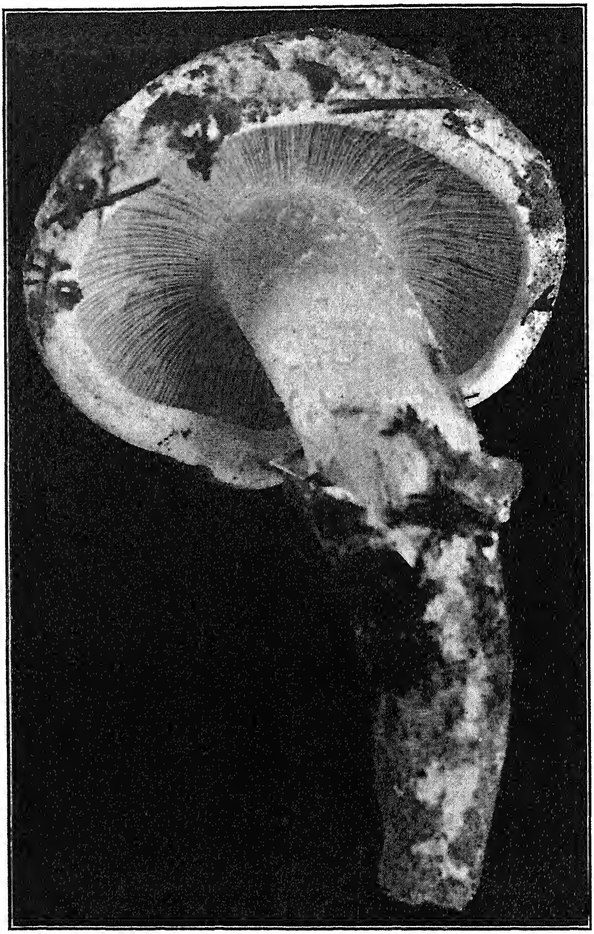


FIG. 4. *Armillaria ponderosa* collected north of Florence, Oregon, November 28, 1932. Photo by S. M. Zeller. $\times \frac{3}{4}$.

Washington: Clellam Co., near Port Angeles, *J. W. Hotson,* Autumn, 1930 (in Univ. Wash. Herb.); Kitsap Co., Bainbridge Island, *J. W. Hotson,* Oct., 1926 (in Univ. Wash. Herb.); San Juan Co., Waldron Island, *Ethan Allen,* Dec. 3, 1932; Whatcom Co., Dot Island, Chuckanut Bay, *Ethan Allen,* Dec. 5, 1932.

Oregon: Hood River Co., toward Mt. Hood above Parkdale, *Katzuko Konishi,* Oct. 22, 1931; Tillamook Co., Nehalem, *C. K. Ogura,* Oct. 2, 1932; Lane Co., near Florence, *Ralph Johnson,* Oct. 16, Nov. 10, 1932, *Mr.* and *Mrs. S. M. Zeller,* Nov. 28, 1932 (collection of over 200 specimens).

COMPARISON OF THE AMERICAN AND JAPANESE MATSU-TAKES

The particular question in connection with *Armillaria ponderosa* on the Pacific Coast is its taxonomic relationship to *A. matsutake* of Japan. The two must be very similar in gross external anatomy, odor, and taste, since the Japanese laymen on both sides of the Pacific Basin believe them to be identical. A few interesting comparisons may be drawn.

TABLE 1

BIOMETRIC DATA FOR LENGTH AND WIDTH OF 400 SPORES OF THE AMERICAN MATSU-TAKE, BASED ON 100 SPORES FROM EACH OF 4 DIFFERENT MATERIALS (IN μ)

Material	Range	Mode	Mean	Standard deviation	Coefficient of variability
Length					
Z 5597......	4.0–7.0	5.0	5.29 ± 0.03	0.58 ± 0.02	11.11 ± 0.53
Z 5595......	4.0–6.0	5.0	4.93 ± 0.03	0.51 ± 0.02	10.44 ± 0.49
Z 8129......	4.0–6.0	5.0	5.14 ± 0.03	0.49 ± 0.02	9.53 ± 0.45
Z 8030......	4.0–7.0	5.0	5.30 ± 0.03	0.51 ± 0.02	9.80 ± 0.46
Total.......	4.0–7.0	5.0	5.16 ± 0.01	0.55 ± 0.01	10.65 ± 0.25
Width					
Z 5597......	4.0–5.0	4.0	4.30 ± 0.02	0.41 ± 0.01	9.58 ± 0.45
Z 5595......	3.0–5.0	4.0	4.08 ± 0.01	0.27 ± 0.01	6.64 ± 0.31
Z 8129......	4.0–5.0	4.0	4.16 ± 0.01	0.26 ± 0.01	6.32 ± 0.30
Z 8030......	4.0–5.0	4.0	4.14 ± 0.01	0.26 ± 0.01	6.44 ± 0.30
Total.......	3.0–5.0	4.0	4.17 ± 0.01	0.32 ± 0.007	7.67 ± 0.18

Materials	Locality	Date	Collector
Z 5597..	Florence, Oregon	Nov. 28, 1932	S. M. Zeller
Z 5595..	Waldron Island, Washington	Dec. 3, 1932	E. Allen
Z 8129..	Lane County, Oregon	Oct. 16, 1932	R. Johnson
Z 8030..	Odell, Oregon	Oct. 22, 1931	K. Konishi

Color.—The American is lighter in color than the Japanese Matsu-take. The usual color of the fibrils and disk of *A. matsutake* when fresh is tawny, russet, or Mars brown, while in *A. ponderosa* it is usually pinkish buff or light ochraceous salmon. In very extreme cases of exposure to rain or direct sunlight after rain, very mature specimens of *A. ponderosa* may show as dark colors as *A. matsutake* usually exhibits. In these extreme cases the colors are almost exactly those of the latter. Figures 2, 4, and 5 show very well the degree of difference in color.

Surface of the pileus.—In *A. matsutake* the surface is characteristically fibrous scaly, the fibrils loose and easily disturbed by rubbing. On this account the surface is often fissured radially toward the margin. On the other hand the surface of *A. ponderosa*

TABLE 2

BIOMETRIC DATA FOR LENGTH AND WIDTH OF 400 SPORES OF THE JAPANESE MATSU-TAKE, BASED ON 100 SPORES FROM EACH OF 4 DIFFERENT MATERIALS (IN μ)

Material	Range	Mode	Mean	Standard deviation	Coefficient of variability
Length					
T 1.........	3.0–8.0	6.0	5.85 ± 0.05	0.80 ± 0.03	13.75 ± 0.65
T 2.........	4.0–8.0	5.0	5.90 ± 0.06	0.95 ± 0.04	16.16 ± 0.77
T 3.........	4.0–6.0	5.0	5.20 ± 0.03	0.52 ± 0.02	10.17 ± 0.48
T 4.........	4.0–6.0	5.0	5.36 ± 0.04	0.60 ± 0.02	11.35 ± 0.54
Total.......	3.0–8.0	5.0	5.57 ± 0.02	0.80 ± 0.01	14.38 ± 0.34
Width					
T 1.........	3.0–6.0	4.0	4.48 ± 0.03	0.53 ± 0.02	12.01 ± 0.57
T 2.........	3.0–5.0	4.0	4.35 ± 0.03	0.55 ± 0.02	12.74 ± 0.60
T 3.........	4.0–5.0	4.0	4.47 ± 0.03	0.49 ± 0.02	11.16 ± 0.53
T 4.........	4.0–5.0	4.0	4.30 ± 0.03	0.45 ± 0.02	10.65 ± 0.50
Total.......	3.0–6.0	4.0	4.40 ± 0.01	0.51 ± 0.01	11.80 ± 0.28

Materials	Locality	Date	Collector
T 1.....	Kadoma, Pref. Iwate	Oct. 11, 1932	From market of Morioka
T 2.....	Kadoma, Pref. Iwate	Oct. 13, 1932	From market of Morioka
T 3.....	Kasugabe, Pref. Hyogo	Oct. 12, 1932	Y. Sato
T 4.....	Minomo, Pref. Osako	Oct. 23, 1932	Y. Sato

is smooth, subviscid so that sand or soil cling, becoming innate fibrillose, but really becoming *fibrous scaly only after rains.*

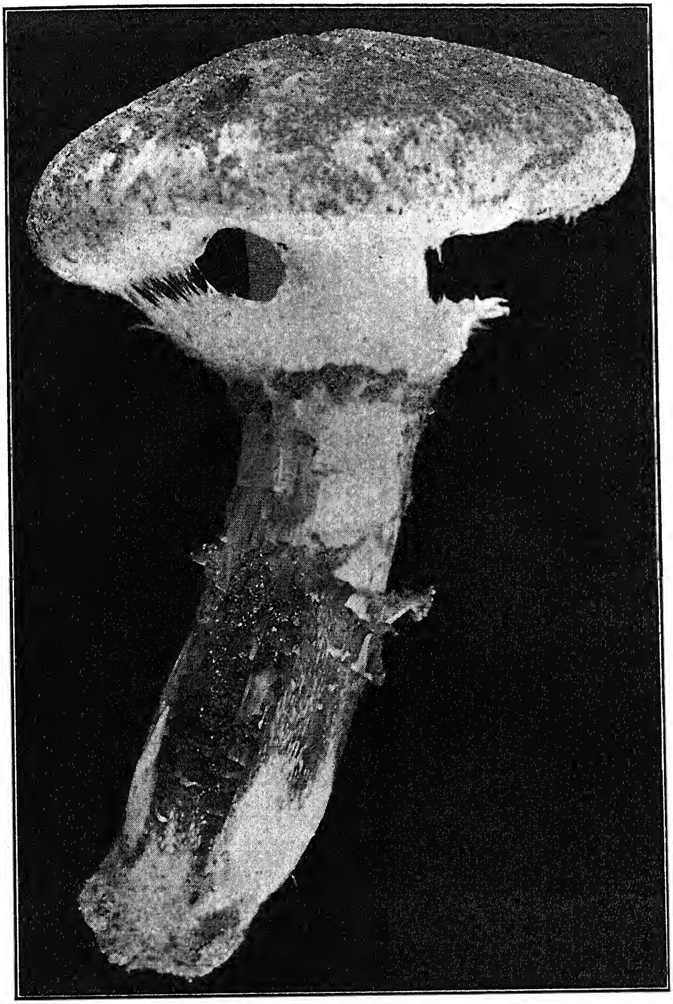


FIG. 5. *Armillaria ponderosa* showing the breaking of the veil, collected north of Florence, Oregon, November 28, 1932. Photo by S. M. Zeller. × ¾.

Spores.—The spores of the two species are extremely close in shape and measurements as tables 1 and 2 clearly show. This biometric study of the spores of the two species indicates that there is no significant difference between spore sizes of the two, even

though the total spores of the Japanese form are somewhat larger than those of the American form.

In this connection a more or less irrelevant note concerning the variability of spore shapes and sizes in subsequent spore-prints from the same pileus may be of interest. Table 3 illustrates the

TABLE 3

BIOMETRIC DATA FOR LENGTH AND WIDTH OF EACH OF 100 SPORES FROM THE SAME PILEUS (*A. matsutake*) (IN MICRONS). MATERIAL: IWATE-GUN, PREF. IWATE, OCT. 11, 1932

Date	Spore-print	3.0	4.0	5.0	6.0	7.0	8.0	Mean	St. dev.	Coeff. var.
								Length		
Oct. 12	1st*	1	2	27	53	15	2	5.85 ± 0.05	0.80 ± 0.03	13.75 ± 0.65
Oct. 13	3rd	1	8	48	41	2	0	5.35 ± 0.04	0.69 ± 0.03	13.05 ± 0.62
Oct. 14	5th	1	7	45	42	5	0	5.43 ± 0.04	0.73 ± 0.03	13.59 ± 0.64
Oct. 16	9th	0	3	58	35	4	0	5.40 ± 0.04	0.61 ± 0.02	11.40 ± 0.54
								Width		
Oct. 12	1st	1	51	47	1			4.48 ± 0.03	0.53 ± 0.02	12.01 ± 0.57
Oct. 13	3rd	5	76	19	0			4.14 ± 0.03	0.46 ± 0.02	11.33 ± 0.54
Oct. 14	5th	1	64	35	0			4.34 ± 0.03	0.49 ± 0.02	11.38 ± 0.54
Oct. 16	9th	0	55	45	0			4.45 ± 0.03	0.49 ± 0.02	11.17 ± 0.53

* Printed twice a day.

point that especially the length of spores varies with maturity of the fructification. The tendency is toward slightly larger spores in the younger stages and somewhat smaller and rounder spores in the later spore-prints, where the variability of spore size also becomes narrower. This tendency toward rounder spores at maturity may partially explain Peck's, as well as Murrill's, observations of globose or subglobose spores in their descriptions (*A. ponderosa, A. magnivelaris,* and *A. arenicola*). This may also explain some of the variability in Table 2.

Ecology.—For the most part the ecology of the two species has been discussed above. It is of particular interest here to note, however, the difference in types of soil which favor the production of each. The Japanese Matsu-take seems to grow most favorably in the Chichibu palaeozoic or decomposed granite soils covered by a thick layer of forest duff and also on mountain slopes having

similar soils. It seldom, if ever, develops in sandy locations as does the American Matsu-take. On the main island (Honshu) of Japan *Pinus densiflora,* the living roots of which play an essential

FIG. 6. *Armillaria ponderosa* showing the characters of the surface of the pileus, collected near Florence, Oregon, Nov. 28, 1932. Photo by S. M. Zeller. × ¾.

rôle in the production of Matsu-take (Masui, 1927), occurs rather commonly in sandy coastal plains, but Matsu-take is not collected in such locations. On the contrary *A. ponderosa* is found at its best and in quantity in the pine (*Pinus contorta*) barrens of the coastal sand dunes and in very sandy loams of the mountain areas (Mt. Hood). The types of hymenomycetous associates of the two are very similar, and both are ectotropic mycorrhizal fungi.

Both species are exceptionally palatable mushrooms, having a firm, fine texture and a distinctively good flavor. Both are highly prized by the Japanese people. Both species are good canners in the button stages, but lose their fragrance in the dried condition,

a sharp contrast with Shii-take (*Cortinellus*) which is used commonly as a dried food in Japan.

According to the usual taxonomic comparisons *Armillaria matsutake* and *A. ponderosa* differ but slightly, especially in morphological characters. They suggest the possibility that wide geographic separation and ecological conditions may produce variants such as these in a single species of Agarics and that it may be necessary in certain groups of the gill fungi to take into consideration certain ecological and physiological conditions in their final classification into species. In this case, however, after a consideration of all of the differences the authors feel that the two are perhaps specific entities.

INDEX TO VOLUME XXVI

INDEX TO AUTHORS AND TITLES

INDEX TO GENERA AND SPECIES

New names and the final members of new combinations are in **bold face type.** Mistakes in the spelling of scientific names encountered in text are corrected in the index.